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STUDIES IN NEUROMUSCULAR FUNCTION

I. Introduction and Methods[1]

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Received for publication March 15, 1956

In recent years much has been learned concerning the mechanisms involved in normal neuromuscular function. Most of this knowledge has been gained from studies in experimental animals. Less attention has been paid to the study of neuromuscular function in man, and to the alterations that occur in disease states. In this series of communications, observations will be presented on the characteristics of the response of skeletal muscle to motor nerve stimulation in normal subjects and in patients with myasthenia gravis. The action of intra-arterially injected acetylcholine (ACh), the mediator of neuromuscular transmission, on these responses will be described, as well as the effects of choline, anticholinesterase compounds, d-tubocurarine and decamethonium. Evidence regarding the nature of the defect in neuromuscular transmission in myasthenia gravis will be presented. In later communications, other aspects of normal and abnormal neuromuscular function will be considered, including the role of potassium ions, the mechanism of weakness in familial periodic paralysis, and the phenomenon of fatigue.

This introductory paper will briefly summarize current concepts concerning neuromuscular transmission and the characteristics of the various types of neuromuscular block that have been described in experimental animals. The methods employed in this series of studies on neuromuscular function in man will also be described.

Muscle Excitation

The resting muscle fiber normally has a potential difference of approximately 90 millivolts (mV) between the two sides of the surface membrane, with the inner surface being negative with respect to the outer (1–3). It is, therefore, said to be in a state of polarization. This resting potential has been attributed mainly to the difference between the intracellular and extracellular concentrations of potassium and sodium ions (4). Stimulation of the muscle fiber indirectly through its motor nerve, or directly by a cathodal (outward) current applied to the fiber, results in the inner surface of the membrane becoming

[1] This work was supported by a contract between the Johns Hopkins University and the U. S. Army Chemical Corps, and was aided by a grant from the Division of Neurologic Diseases and Blindness, National Institute of Health, U.S.P.H.S.

less negative; in fact, it becomes approximately 10 mV positive with respect to the outside of the fiber. The cell membrane is said to have become "depolarized", although it actually becomes polarized in the reverse direction. There is evidence (4) that this is due to a temporary increase in permeability of the nerve or muscle membrane to sodium ions, which enter the cell from the extracellular fluid, resulting in the inner surface of the membrane becoming more positive, (or the outer surface more negative, depending upon which is chosen as a reference). This localized area of depolarization produces an outward current flow in the adjacent polarized membrane, resulting in its depolarization. Thus, a wave of depolarization is propagated along the muscle membrane. It is believed that repolarization is accomplished initially by an outward flow of potassium ions followed by an active process in which sodium ions are removed from the cell and potassium ions are restored to the cell. The muscle fiber is then again ready for excitation. This propagated wave of depolarization and repolarization is known as the muscle action potential. While the spread of depolarization is intimately associated with the initiation of muscular contraction, the exact mechanism is not presently clear.

Neuromuscular Transmission

The resting muscle fiber exhibits at the end-plate, the specialized region of the membrane adjacent to the motor nerve endings, constantly recurring minute potentials which are localized and non-propagated (6). These miniature end-plate potentials are attributable to the release from the motor nerve endings of subliminal quanta of acetylcholine (ACh). This compound diffuses to the adjacent end-plate, where it combines momentarily with receptor substance. ACh has the property of depolarizing the muscle membrane in the region of the end-plate. The miniature end-plate potentials produced are not of sufficient amplitude to depolarize the muscle membrane around the end-plates and initiate a propagated muscle action potential. However, when a nerve impulse reaches the motor nerve endings, a larger amount of ACh is released which produces an end-plate potential of sufficient amplitude to depolarize the muscle membrane around the end-plate (7). This initiates the muscle action potential, which is propagated as a wave of depolarization along the muscle fiber surface and is in turn followed by shortening of the contractile elements. The injection of ACh or its application on the muscle fiber at any place other than the end-plate has no appreciable effect on the membrane potential.

Shortly after ACh initiates depolarization of the end-plate, it is hydrolyzed into acetate and choline by the enzyme cholinesterase concentrated in this region. The potential difference across the muscle membrane is then restored, beginning at the end-plate; i.e. repolarization occurs and the end-plate and remainder of the muscle membrane are again ready to respond to another nerve impulse.

Muscle Action Potential Response to Nerve Stimulation

Each motor nerve fiber innervates a number of muscle fibers, varying from one to five in the extraocular muscles, where fine control is needed, to about two hundred in large muscles, where power is more important. Each motor neurone and the muscle fibers which it innervates constitute a motor unit. Since each nerve or muscle fiber responds to excitation in an all-or-none manner, a motor unit normally responds to a single stimulus in the same way. When a single electrical stimulus of sufficient strength and duration is applied to the motor nerve a single muscle twitch is elicited. Each nerve fiber that is stimulated produces depolarization and contraction of the muscle fibers which it innervates. As the strength of stimulus is increased more nerve fibers and hence more motor units respond, so that the muscle action potential and strength of contraction of the muscle increase. A nerve stimulus that excites all the nerve fibers, and hence all the muscle fibers supplied by that motor nerve, is called a maximal stimulus. A further increase in stimulus intensity does not produce an increase in muscle response, since all the motor units are already being activated. The muscle action potential produced by nerve stimulation, which can be recorded by placing an electrode on the skin over the muscle, or in the muscle itself, represents an integration of the action potentials of the individual muscle fibers in the region of the recording electrode (5). When the nerve stimulation is maximal, or supramaximal, the amplitude of the integrated action potential, (or, more precisely, the area under the potential), is a function of the number of muscle fibers responding to the nerve stimulus.

Causes of Neuromuscular Block

Disturbance of neuromuscular transmission may result from interruption of the normal sequence of events at any step. The various types of neuromuscular block will be listed and their characteristics briefly discussed.

I. Deficient release of transmitter substance (ACh) from the motor nerve endings. This is believed to take place in botulinus poisoning (8), and in severe hypocalcemia in the experimental animal (9).

II. Excessively rapid removal of transmitter following its release from the nerve endings. This could result from an excessive local concentration of muscle cholinesterase, but such a mechanism has not been observed or experimentally reproduced.

III. Inhibition of the depolarizing action of the transmitter on the end-plate. This may be termed an "*ACh-inhibitory block*", of which there are three types:

A. Block occurring without change in the resting potential of the muscle membrane, and reversible by ACh or an anticholinesterase compound. The block produced by d-tubocurarine in experimental animals is of this type (10, 11). This drug is believed to exert its effect by competing with ACh for receptor

sites at the end-plate, so that the resulting block has been termed "*competitive*". This type of block has the following characteristics:

1. It is not preceded by an increase in motor activity.

2. There is a progressive decline in the muscle action potentials in response to two or more nerve stimuli. Since competitive blocking agents such as d-tubocurarine do not affect the output of ACh from the motor nerve ending, it is presumed that the decline in the response to repetitive nerve stimuli reflects variations in the amount of ACh normally liberated (12, 13). In the absence of block there is no such decline, since the decreasing amounts of ACh liberated with repeated activity remain sufficient to depolarize all the end-plates. Curarization, by raising the threshold of the end-plates to the stimulating action of ACh, reveals the progressive decline in the amount of transmitter released with repetitive nerve stimulation.

3. There is an increase in the muscle action potential response to a single nerve stimulus delivered after tetanic stimulation; i.e. post-tetanic facilitation. Observations on post-tetanic decurarization in experimental animals have indicated that this is due to increased output of transmitter substance (ACh) from the nerve endings, as reflected by an increase in the end-plate potential (11, 12). When a train of stimuli is delivered after tetanic stimulation, the response to the first stimulus of the train is increased, but subsequent responses are of lower amplitude than those of the initial train. This appears to be due to decreased output of transmitter from the nerve endings (13).

4. There is a decrease in the depolarizing action of injected ACh or anticholinesterase compound (10).

5. The block is reversed following the injection of sufficient ACh or anticholinesterase compound (11).

B. Block not associated with any change in membrane potential, but not reversible by ACh. This type of block is reported to occur several minutes after the exposure of frog muscle to ACh (14).

C. Block associated with hyperpolarization of the membrane. An example is the block produced in the experimental animal by the application of an anodal current to the end-plate region. There is evidence that the defect in familial periodic paralysis is due in part to this mechanism, resulting from an increase in the gradient of intracellular to extracellular concentration of potassium (15).

IV. Abnormally prolonged depolarization in the region of the end-plates. When this region is in the depolarized state, it becomes inexcitable. This type of block is produced by an excessive concentration of ACh (1, 16), by anticholinesterase compounds (1), and, in most animals species, by decamethonium (16) or choline (17). It has the following characteristics:

1. It is preceded by an increase in motor activity, due to the initial end-plate depolarization.

2. There is no progressive decline in the muscle action potentials in response to two or more nerve stimuli, except following anticholinesterase compounds,

which cause accumulation of ACh and intensification of the block following each stimulus.

3. There is no post-tetanic facilitation.

4. There is no decrease in the depolarizing action of injected ACh or anticholinesterase compound, so that these have an additive effect.

5. The block may be reversed by the injection of a competitive blocking agent, such as d-tubocurarine, but not by ACh or an anticholinesterase compound (16).

Neuromuscular block which is non-ACh-inhibitory and non-ACh-reversible (i.e. "*non-competitive*") is frequently presumed to be of the depolarzing type, but demonstration of such a mechanism requires measurement of the membrane potential in the region of the end-plate. This measurement has not yet been carried out in man.

V. Mixed types of block. Precise characterization of neuromuscular block may be rendered difficult by the occurrence of properties of more than one of these types, or by change in the type of block with time. Thus, following the administration of decamethonium to the monkey, dog, or rabbit, the resulting neuromuscular block has the properties of an ACh-reversible (competitive) type, but it is preceded by increased motor activity suggestive of initial depolarization (18). Furthermore, different muscles may not respond in the same manner. In the cat, the block produced by decamethonium in the tibialis and gracilis, which are pale muscles, is of the depolarizing type, while in the soleus, a red muscle, it appears to be initially depolarizing and then competitive (19).

Method of Study

In the observations to be reported an attempt has been made to describe the properties of the neuromuscular block present in patients with myasthenia gravis, and the properties of the block produced in normal subjects and in myasthenic patients by various chemical agents. The presence or absence, and degree, of the following features of the neuromuscular block have been investigated:

1. Increased motor activity, as indicated by muscle movement and potentials occurring in the absence of prior nerve stimulation.

2a. Reduction of the muscle action potential response to a single supramaximal nerve stimulus.

b. Progressive decline in the muscle action potentials in response to two or more nerve stimuli.

3. Post-tetanic facilitation and more rapid decline in the response to subsequent stimuli of the second of two tetani.

4. Inhibition of the "depolarizing" action of ACh and of anticholinesterase compounds.

5. Reversal of the block by ACh and by anticholinesterase compounds.

Recording of Muscle Action Potentials

The method of Harvey and Masland (20) was employed in which muscle action potentials are recorded from the *abductor digiti quinti* after stimulation of the ulnar nerve. Muscle action potentials produced in this manner will be referred to as evoked or induced potentials. To elicit them, the nerve was stimulated percutaneously just above the elbow with supramaximal rectangular pulses of 150 microseconds duration. The pulses were delivered from a two channel stimulator through an ungrounded isolation transformer. The stimulating cathode was either an electrode paste bridge or a brass rod of 5 millimeters diameter. It was essential to strap the stimulating cathode securely to the arm to prevent movement of the electrode with respect to the nerve during repetitive nerve stimulation. Small movements of the stimulating electrode may result in submaximal nerve stimulation even when the stimulus intensity was initially several times maximal. The indifferent anode was a 4 by 6 centimeter plate strapped to the ulnar side of the forearm. Good electrical contact was obtained with commercial electrode jelly.

Recording electrodes (7 millimeter silver discs) were attached with bentonite-potassium chloride paste to the skin over the belly of *m. abductor digiti quinti* and over its tendon at the base of the fifth finger. Action potentials were amplified by a differential input, resistance-capacitance coupled amplifier. Potentials were photographically recorded from a dual beam cathode ray oscilloscope. The overall frequency response was flat within 3 decibels from 0.5 to 10,000 cycles per second. A ground strap just above the wrist greatly reduced the size of the stimulus artifact. Movement artifact was minimized by firmly clamping the subject's hand between rubber padded metal plates.

The muscle action potential recorded in this manner was polyphasic. The belly electrode first became negative and then positive with respect to the tendon electrode. This action potential represented an integration of the action potentials of the individual muscle fibers in the region of the recording electrode. When nerve stimulation was maximal and the size of the individual muscle fiber action potentials was constant, the area of the integrated action potential was a function of the number of muscle fibers responding to the nerve stimulus. Therefore, extreme care was taken to insure maximal nerve stimulation. Using a submaximal stimulus intensity a careful search was made with the stimulating electrode for the point at which muscle action potentials of greatest amplitude were evoked. The stimulus intensity was then increased to a level 50 per cent greater than that which produced action potentials of maximum size. This will be called supramaximal nerve stimulation.

The initial deflection of the action potential has been recorded in an upward direction. The amplitude (height) of this initial negative deflection above the base line was measured by projecting the photographic record on a ruled

screen. Since alterations in action potentials which occurred consisted mainly of alterations in amplitude, with much less change in duration, the amplitude was considered to be approximately proportional to the area of the action potentials and, therefore, to the number of muscle fibers responding to the nerve stimulus.

Patterns of Nerve Stimulation

To study decline in the muscle action potentials in response to two nerve stimuli, a second (test) stimulus was delivered at intervals of 16 msec. to 10 seconds after an initial (conditioning) stimulus. To study the response to repetitive nerve stimuli, these were generally delivered at 40 msec. intervals in trains of from four to several thousand. Post-tetanic facilitation was investigated by delivering to the nerve a test stimulus at intervals of 16 msec. to 15 seconds after a conditioning tetanus. The frequency of stimulation during the tetanus was from 25 to 200 per second, and the duration of stimulation was one half second to two minutes. In some instances another tetanus was substituted for the test stimulus.

In order to observe the time course of the effect of drugs on the muscle action potential response to nerve stimulation, these were injected into the brachial artery during intermittent stimulation of the ulnar nerve. Except where otherwise indicated, the standard pattern consisted of trains of four stimuli (interval between each stimulus 40 msec.) delivered every 2 to 10 seconds. The injection was usually made after the fourth such train, so that a suitable period of response to nerve stimulation was recorded as a control.

Injection of Drugs

The intra-arterial administration of drugs during intermittent supramaximal nerve stimulation has provided a useful method for the study of their effects on neuromuscular transmission, and the time course of these effects. By this route, amounts could be given which resulted in profound changes in function in the injected extremity without producing systemic manifestations. This greatly increased safety, enabled a wider range of concentration to be studied, and facilitated the evaluation of antagonistic and synergistic actions of various drugs. Intra-arterial administration was essential in studying the neuromuscular effects of ACh, since this drug is rapidly hydrolyzed in the blood stream.

In the studies to be reported, the drugs injected intra-arterially were acetylcholine chloride (ACh) (Merck), neostigmine methylsulfate (Prostigmine, Hoffmann-La Roche), atropine sulfate, sodium acetate, choline chloride (Squibb), edrophonium chloride (Tensilon, Hoffman-La Roche), d-tubocurarine chloride (Intocostrin, Squibb), and decamethonium chloride (C_{10}, Syncurine, Burroughs-Wellcome). The solutions of ACh were freshly prepared for each procedure. The volume of injection was 2 ml. in all instances, with dilu-

tions made with isotonic saline solution. All doses have been expressed in milligrams of the above mentioned salts.

All injections were made into the brachial artery through an inlying 20 gauge Cournand-Riley needle, except that atropine was usually administered intramuscularly prior to the injection of ACh or neostigmine for protection against the muscarine-like effects of these drugs. A blood pressure cuff was placed about the upper arm. Immediately preceding each intra-arterial injection, the needle was checked for patency, and the pressure in the cuff was then raised to 300 mm. Hg, occluding the artery. The injection was made as rapidly as possible, and the pressure in the cuff immediately released. Then, the pressure in the cuff was raised to just above venous pressure for 2 to 3 minutes in order to retard escape of the drug into the systemic circulation.

During the course of each experiment several factors which may introduce variability in the effect of injected drug on neuromuscular transmission were controlled. Care was taken to insure that the position of the stimulating electrode did not change and that nerve stimulation was supramaximal at all times. Adequate time was allowed for recovery from the effects of each period of nerve stimulation. Variations in blood flow through the injected extremity were minimized, as these may influence dose-effect relationships. Since increased blood flow may occur as a result of muscle activity, excitement, ingestion of food, or the prior injection of a vasodilator drug, the patient was studied in a basal condition and successive injections were appropriately spaced. Thus, ACh was injected at intervals of more than ten minutes, since this is the maximal period of flushing and increased blood flow following the administration of 5 mg. of this drug into the brachial artery (21). Differences in arm volume no doubt influence the dose-effect relationship of intra-arterially injected drugs, but correction for this factor did not decrease the range or standard deviation of the effect of drugs such as ACh, indicating that other variables are more important. Occasionally anomalies of the arterial tree were encountered in which the ulnar artery branched from the brachial artery proximal to the injection site. This was detected by the absence of flushing and sweating over the ulnar aspect of the arm and hand following the injection of vasodilator drugs, and the needle was reinserted more proximally.

Patients with Myasthenia Gravis

The patients with myasthenia gravis who were studied have been divided into two groups, those with generalized myasthenia, in whom the upper extremities, including the muscles of the hands, were affected by the disease, and those with oculobulbar myasthenia, in whom the extremities were not affected. The patients with generalized myasthenia had weakness of the arms and hands which improved following the intramuscular or intra-arterial administration of neostigmine. The electromyogram in these patients had the

characteristic defect of myasthenia gravis (22), which improved following the intra-arterial injection of suitable doses of neostigmine (23). The patients with oculobulbar myasthenia had weakness of the extraocular muscles, in most instances weakness of the facial muscles, particularly the orbicularis oculi, and in some instances weakness of the muscles of chewing, swallowing, speech, and of the neck. The strength of these muscles improved after the administration of neostigmine. The electromyogram, including the muscle action potential responses to prolonged repetitive nerve stimulation, recorded from *m. abductor digiti quinti*, was normal, and intra-arterially injected neostigmine produced depression of these responses, as in normal subjects (24).

SUMMARY

Current concepts of the mechanism of normal neuromuscular transmission and of the various types of neuromuscular block have been summarized, and the methods to be employed in this series of studies described.

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STUDIES IN NEUROMUSCULAR FUNCTION

II. Effects of Nerve Stimulation in Normal Subjects and in Patients with Myasthenia Gravis[1, 2]

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Previous observations have revealed several abnormalities of neuromuscular transmission in myasthenia gravis. There is partial block to the passage of a single impulse across the neuromuscular junction (2). This block is increased after a single impulse traverses the neuromuscular junction (3). Repetitive nerve stimuli evoke successively smaller muscle action potentials, indicating an increasing block in neuromuscular transmission (3, 4). These abnormalities are similar to those observed in the normal subject during a partial block in neuromuscular transmission produced by d-tubocurarine (5, 6).

In this communication a more detailed study of these and other abnormalities in neuromuscular function has been made in order to obtain further information as to the nature of the defect in myasthenia gravis. The response to tetanic motor nerve stimulation, the time course of the increased block following the passage of a single nerve impulse, and the phenomenon of post-tetanic facilitation of transmission observed in the present studies are also similar to the changes produced in normal subjects by partial curarization (6).

The methods employed in this study have been described (7). Five types of stimulus patterns were used. First, the muscle action potential response to a single supramaximal nerve stimulus was recorded. Second, the effect of passing a single impulse across the neuromuscular junctions upon their ability to transmit a subsequent impulse was tested. A single conditioning stimulus was followed after varied time intervals by a second, or testing, stimulus. The amplitude of the muscle action potential response to the testing stimulus was compared with the amplitude of the first, or control response. Third, the response to repetitive (tetanic) stimulation of the motor nerve was observed by applying trains of stimuli of different frequency for different lengths of time. Fourth, the effect of tetanic stimulation on the ability of the neuromuscular junctions to transmit a subsequent impulse was detected by applying a conditioning train of stimuli followed by a single test stimulus at different time

[1] This work was supported by a contract between the U. S. Army Chemical Corps and The Johns Hopkins University, and was aided by a grant from the Division of Neurologic Diseases and Blindness, National Institute of Health, U.S.P.H.S.

[2] Part of this study has been presented in a preliminary report (1).

intervals. The test response was compared in amplitude with a control single response; often the first response to the conditioning train was used as a control. Fifth, the effect of tetanic stimulation on the ability to transmit a second tetanus was similarly studied by delivering conditioning and testing trains of stimuli separated by varying time intervals.

RESULTS

A. The muscle action potential response to a single supramaximal nerve stimulus

1. In normal subjects: In 20 subjects the mean amplitude of the muscle action potential produced by a single nerve stimulus was 7.5 millivolts (mV). The standard deviation was 2.4 mV and the standard error of the mean 0.55 mV. The amplitude of response in a given experiment on a normal subject was remarkably constant and did not vary during the day.

2. In patients with oculobulbar myasthenia gravis: The mean amplitude of the action potential in response to a single nerve stimulus in a group of 9 patients was 5.9 mV (standard deviation 1.6 mV, standard error of mean 0.53 mV). The mean amplitude in this group is lower than the mean amplitude of the control group, but the difference is barely significant ($p < 0.05$).

3. In patients with generalized myasthenia gravis: Twenty-six patients were studied, and the mean amplitude of their response to a single maximal nerve stimulus was 5.4 mV (standard deviation 2.9 mV, standard error of mean 0.56 mV). The difference between this mean, and the mean amplitude in the normal group is significant ($p < 0.01$). However, the potential in 22 of these 26 patients lay within the normal range (normal mean ± 2 S.D.).

Progressive fall in amplitude of response to a single stimulus was observed to occur during the course of the day in some patients with severe involvement of the hand muscles. The amplitude was restored to the original level after a night's rest. A similar persistent fall occurred more rapidly following a series of nerve tetani. Repeated series of tetani applied to the ulnar nerve in one arm did not produce any alteration in the response to stimuli applied to the opposite ulnar nerve.

B. The effect of the transmission of a single impulse across the neuromuscular junction on its ability to transmit a second impulse

Single conditioning and testing nerve stimuli were delivered. The interval between the two stimuli was varied between 16 msec. and 10 sec.

1. In normal subjects: The amplitude of the muscle action potential in response to the test stimulus equalled that of the control (conditioning) response over the entire range of intervals tested. At intervals of less than 16 msec. the electrical responses began to fuse, making simple amplitude measurements unreliable.

2. In patients with oculobulbar myasthenia gravis: The amplitude of the two responses was equal, as in the normal subjects.

3. In patients with generalized myasthenia gravis: Unlike the normal response, as the interval between the two stimuli was increased from 16 msec. to 0.5 or 1.0 sec. the second response decreased in amplitude (Figs. 1A and 2A). As the interval was increased beyond 1.0 sec. there was gradual increase in amplitude, but it was not until the interval between stimuli reached 10 sec. that it again equalled that of the first.

C. The muscle action potential response to repetitive (tetanic) nerve stimulation

The nerve was stimulated at frequencies of from 1 to 200 per sec. The duration of the train of stimuli was usually 2 minutes.

1. In normal subjects: When stimuli were delivered repetitively at rates from one through 25 per sec. for periods up to one minute the successive muscle action potential responses remained equal in amplitude to that of the initial response. When the repetitive nerve stimulation was continued for two minutes the responses were well maintained at rates of 1 through 10 per sec., but at a rate of 25 per sec. the muscle action potentials decreased slightly in amplitude and increased slightly in duration. When the stimuli were delivered at a rate of 50 per sec. or faster these changes became more marked.

2. In patients with oculobulbar myasthenia gravis: The response did not differ from that seen in the normal subjects.

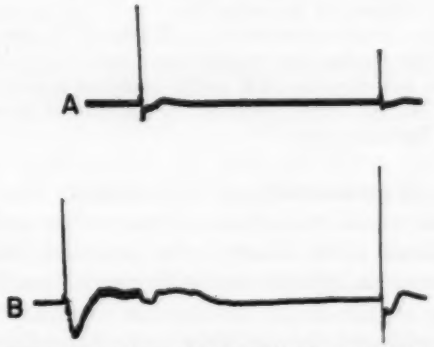

FIG. 1. Electromyogram in a patient with generalized myasthenia gravis (R. S.) showing muscle action potentials in response to maximal nerve stimuli.

A, depression of response to a single test stimulus delivered 2 sec. after a single conditioning stimulus. The response to the latter measures 5 mV.

B, post-tetanic facilitation of the response to a single test stimulus delivered 2 sec. after a conditioning train of 121 stimuli at 200 per sec. There are action potentials in response to only the first three stimuli at 200 per sec. The period of stimulation can be seen as a broadening of the baseline produced by stimulus artifact.

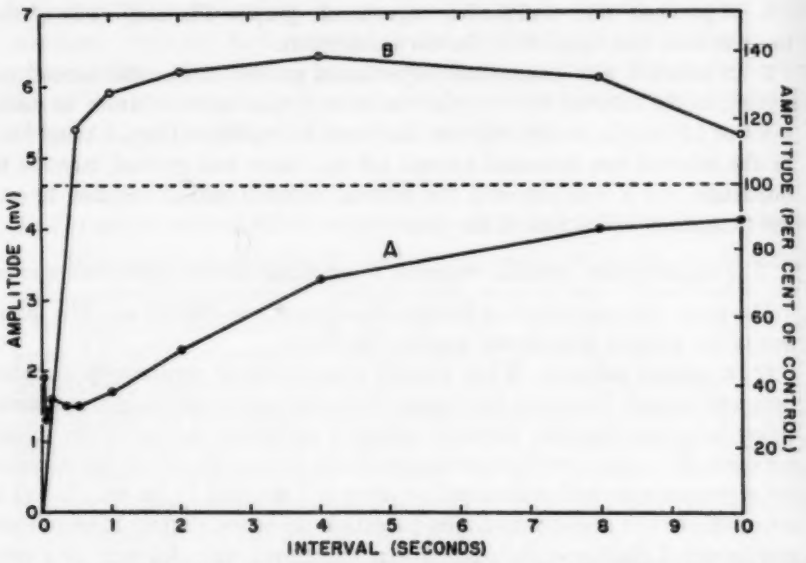

FIG. 2. A, graph of the time course of the depression of the muscle action potential response to a single test stimulus applied at different intervals after a single conditioning stimulus in a patient with myasthenia gravis (R. S.). The amplitude of the second response (closed circles) is plotted in both millivolts and per cent of control response against the time-interval between the stimuli. The amplitude of the control response (response to the conditioning stimulus) is indicated by the broken line.

B, graph of the time course of post-tetanic facilitation of the test response in the same myasthenic patient. The conditioning stimulus was a train of 121 stimuli at 200 per sec. The amplitude of the test response (open circles) is plotted against the time interval between the end of the train and the test response. The amplitude of the control single response is again indicated by the broken line.

3. In patients with generalized myasthenia gravis: There was an abnormal response to repetitive nerve stimulation at rates as low as 5 per sec. (Fig. 3). Initially the amplitude of the muscle action potentials declined rapidly and then returned toward the initial value. This was in turn followed by a slow exponential fall in amplitude. As the stimulus frequency was increased the initial trough deepened and the rate of the late exponential fall was increased.

D. The effect of the transmission of a train of repetitive impulses across the neuromuscular junction on its ability to transmit a single impulse

A conditioning train of repetitive nerve stimuli was followed after a time interval by a single testing nerve stimulus. The frequency of the repetitive stimuli was varied between 5 and 200 per sec. The duration of the train of stimuli ranged from 160 msec. to 1 min. The test stimulus was applied from 16 msec. to 15 sec. after the end of the train.

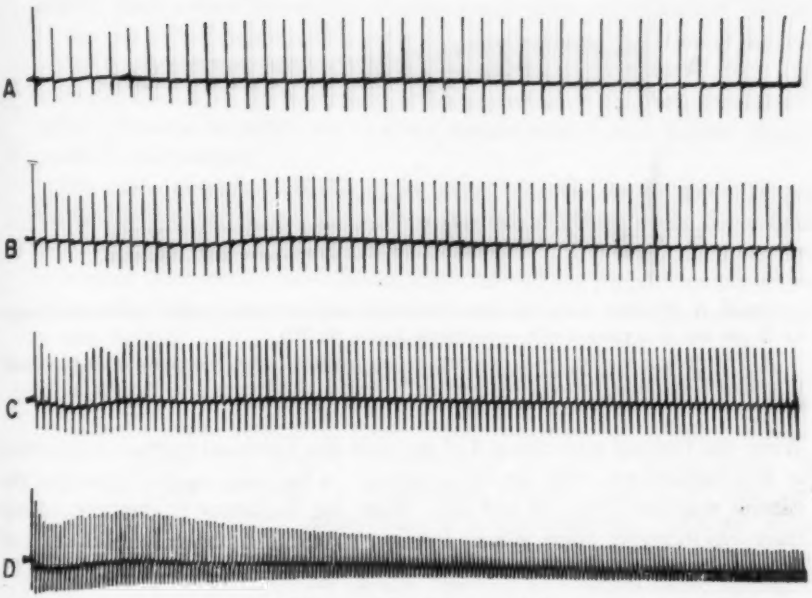

FIG. 3. The characteristic fall in muscle action potential response to repetitive nerve stimulation in a patient with generalized myasthenia gravis (S. P.). Stimulation frequency in A 5 per sec., in B 10 per sec., in C 25 per sec., and in D 50 per sec. The response to the initial stimulus measures 6.2 mV.

1. In normal subjects: When the muscle action potential responses to the train of repetitive nerve stimuli remained constant in amplitude (at stimulation rates of 25 per sec. and below) the amplitude of the response to the test stimulus remained normal at all test intervals. When the amplitude of response to the train of stimuli declined (at stimulation rates over 25 per sec.) the amplitude of the response to the single stimulus was also decreased, but returned to normal within one sec. In no instance did the size of the test response exceed that of the muscle action potential evoked by a single nerve stimulus in the control.

2. In patients with oculobulbar myasthenia gravis: The response of these patients was indistinguishable from normal.

3. In patients with generalized myasthenia gravis: At short intervals the action potential in response to the test stimulus was reduced in size (Fig. 2B). However, as this interval was lengthened the test response increased in amplitude. Usually when the interval between the end of the train of stimuli and the single test stimulus was 0.5 to 1.0 sec. the test response was as large as a control single response. This is to be contrasted with the 10 sec. required for the test response to equal the initial response after the passage of a *single* impulse.

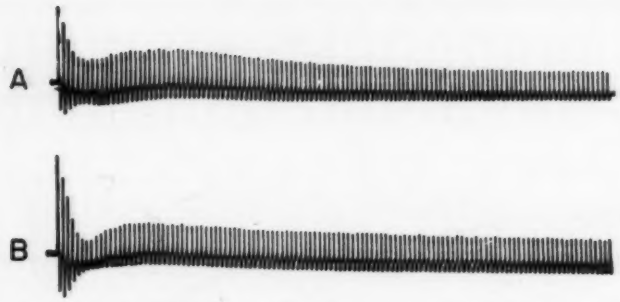

FIG. 4. A. The fall in muscle action potential response to repetitive nerve stimulation at 50 per sec. in a patient with myasthenia gravis (R. P.).

B. Post-tetanic facilitation of the first action potential when the same train of stimuli is applied 5 sec. later, followed by more rapid decline of subsequent potentials.

When the interval after the end of the train was increased further, facilitation of transmission was seen, for the amplitude of the test response exceeded the control response (Figs. 1B and 2B). When the frequency or duration of the train was increased there was an increase in both magnitude and duration of this post-tetanic facilitation.

E. The effect of the transmission of a train of repetitive impulses across the neuromuscular junction on its ability to transmit a second train of impulses

A train of nerve stimuli was followed after an interval by an identical train. The frequency of stimulation was varied from 5 to 200 per sec. The trains durations were 0.5 sec. The interval between the end of the conditioning train of stimuli and the beginning of the testing train was varied from 0.5 sec. to 10 sec.

1. In normal subjects: The muscle action potentials in response to the two trains were identical.

2. In patients with oculobulbar myasthenia gravis: Again, the response to the two trains was identical.

3. In patients with generalized myasthenia gravis: The time course of the response to the first stimulus of the test train paralleled the response to a single test stimulus and exhibited post-tetanic facilitation, as was expected. However, subsequent stimuli in the test train evoked smaller responses than analogous ones in the conditioning train (Fig. 4).

DISCUSSION

The muscle action potential evoked by a single supramaximal nerve stimulus varied widely among normal subjects. This wide variation is to be expected, since, with changes in electrode position or with individual differences in

muscle mass, a variable number of single muscle fiber potentials will contribute to the integrated muscle action potential. Furthermore, variation in the shunting of the action potential by parallel impedances of muscle and other tissues will alter the size of the integrated action potential. In contrast, the size of the action potential recorded from a given normal subject with a given electrode position was constant.

The mean amplitude of the single action potential in the group of myasthenic patients was significantly lower than in normal subjects. Nevertheless, in only four of the 26 myasthenic patients was it clearly below the lower limits of normal. Such statistical evidence is not very strong support for the presence of a partial block of neuromuscular transmission to a single nerve impulse in this disease.

The fact that during the post-tetanic period there was an increase in size of the action potential in response to a single nerve stimulus in patients with myasthenia gravis, including those whose potentials fell within the normal range, indicates that there is recruitment of additional muscle fibers. That there are fibers available for recruitment indicates that there was partial neuromuscular block to transmission of a single impulse. Such an increase was never seen in a normal subject. Neostigmine and allied drugs are capable of increasing the size of the muscle response to a single nerve stimulus in myasthenic subjects, in contrast to the absence of effect or slight decrease in normal subjects (2, 6, 8). Again, this evidence indicates that in the myasthenic subject all of the muscle fibers do not respond to a single supramaximal nerve stimulus. Therefore, while the amplitude of the action potential in a patient with myasthenia gravis may fall within the wide range of normal values, these potentials are capable of being increased by physiological or pharmacological means, thus indicating the presence of some degree of block to neuromuscular transmission.

The lower mean amplitude of single action potentials in the group of patients with oculobulbar myasthenia gravis, as compared with the normal group, is of questionable significance, in view of the normal response of this group to various patterns of nerve stimulation. However, there are demonstrable abnormalities in the response to certain drugs (8, 9), indicating that there may be some abnormality in clinically uninvolved muscles of myasthenic patients.

In patients with severe generalized myasthenia gravis the response to a single nerve stimulus decreased during the course of the day, indicating a progressive increase in the degree of neuromuscular block. This observation cannot be explained by waning of residual neostigmine effect, for all patients were studied at least twelve hours after the last dose of this drug.

A long-lasting increase in the degree of neuromuscular block was also seen after prolonged periods of nerve stimulation in patients with even mild general-

ized myasthenia gravis. That this is a local phenomenon is evidenced by the fact that prolonged stimulation in one arm did not reduce the amplitude of response to test stimuli in the other arm.

In the patients with generalized myasthenia gravis the passage of a single impulse across the neuromuscular junction was followed by a transient increase in the degree of block (Fig. 2A). The time course of this phenomenon has been described previously, but was not followed to complete recovery (2, 10). These observed results differ from a single exponential type recovery curve in that the degree of block is maximal between 0.5 and 1.0 sec. after the passage of the impulse, rather than immediately. These observed curves have been described as a composite of two processes, one of which results in a facilitation of transmission, the other a block of transmission of considerably longer duration (10).

The time course of these changes in neuromuscular transmission following the passage of a single nerve impulse are not unique to the block of neuromuscular transmission in myasthenia gravis. Similar results have been observed in the block produced, for example, by d-tubocurarine in animals (11) and man (5, 6), although the factors responsible may not be the same as in the myasthenic patient. It is not clear from this type of evidence whether the increased degree of block to the transmission of the second impulse is due to prejunctional factors (decreased synthesis or release of transmitter substance), or to post-junctional factors (elevated threshold to transmitter substance or failure of the end-plate potential to give rise to a propagated action potential).

The progressive increase in the degree of neuromuscular block with repetitive stimulation of the nerve is probably the best single measure of the defect in neuromuscular transmission in myasthenia gravis. While this type of observation does not furnish direct evidence, it is interesting to consider the basis for such a characteristic time course in the responses to repetitive nerve stimulation. First, it might reflect the amount of acetylcholine liberated by the nerve endings. A similar time course has been demonstrated in experimental animals during curarization (11–13). Hutter has observed such a prompt fall and slow rise followed by slow progressive fall in end-plate potentials during repetitive stimulation in the fully curarized experimental animal (12). Changes in response are not seen in normal subjects, presumably because the amount of acetylcholine liberated, while variable, is still more than sufficient to give rise to muscle fiber action potentials. Thus, if the myasthenic response is to be explained on this basis it is necessary to assume that the output of acetylcholine is abnormally low, or that there is an elevation of the threshold of the end-plate to acetylcholine action, i.e. a competitive or curare-like block. A second possibility is that these variations in response reflect phasic post-junctional changes. For example, the variation might be produced by changes in end-plate reactivity due to liberation of a blocking substance. As was stated

previously, this type of experiment cannot distinguish between these possibilities or a combination of both.

In the patients with generalized myasthenia gravis post-tetanic facilitation of neuromuscular transmission was readily demonstrated. The period of facilitation of transmission, which had a duration of longer than ten sec., was preceded by a period of increased block of transmission which lasted about one sec. The degree and duration of this facilitation were increased as the length and frequency of the conditioning train were increased, even though no electrical response from the muscle was detected after the first 20 or 30 stimuli at the higher frequencies. Thus, increased stimulation at frequencies above that at which the muscle fibers could respond, or continued stimulation after the muscle fibers ceased to respond, was capable of increasing the degree of facilitation. These facts suggest that the facilitation is a prejunctional (nerve ending) rather than a post-junctional (muscle fiber) phenomenon. In addition, the time course and the relationship to train frequency and train duration of this phenomenon closely parallel that of post-tetanic decurarization in man (6). Animal studies on post-tetanic decurarization by Hutter (14) and Liley and North (13) have shown it to be prejunctional in origin, resulting from an increased output of transmitter substance (ACh) by the nerve endings and not from increased sensitivity of the end-plates to ACh.

This post-tetanic decrease in the myasthenic block is referred to as post-tetanic facilitation of neuromuscular transmission to clearly distinguish it from the post-tetanic potentiation of Von Euler and Brown (15), which has been described in man by Botelho and Cander (16). This latter phenomenon is muscular in origin, is not associated with an increase in muscle action potential, has a much more prolonged time course, and is seen in the normal subject.

When a train of stimuli was substituted for the single test stimulus, the first response to the test train exhibited post-tetanic facilitation, but subsequent responses were of lower amplitude than the corresponding responses to the initial, conditioning train. Thus, the facilitation of transmission of the first response appeared to be at the expense of an increased block to transmission of subsequent impulses. If, as suggested, the facilitation is due to increased transmitter output, the subsequent increased block may be due to decreased transmitter output by the nerve endings.

There is, thus, a rather marked similarity between the muscle action potential responses to various patterns of nerve stimulation in this naturally occurring disease of man and those seen in the experimental animal as a result of partial curarization. It has been demonstrated that there is no effect of curare on the release of transmitter substance. It would then seem reasonable to conclude that in myasthenia gravis there is at least no qualitative defect in the release of transmitter substance. This would suggest that the underlying difficulty may be a quantitative one with respect to synthesis or release of

transmitter substance or an alteration in the responsiveness of the end-plate to this substance. Data relating to these possibilities will be presented in subsequent communications.

SUMMARY

Neuromuscular transmission in normal subjects, patients with oculobulbar myasthenia gravis, and generalized myasthenia gravis was studied by applying a variety of stimulus patterns to the nerve and recording the electrical response of the muscle.

The only abnormality in neuromuscular transmission in the clinically uninvolved muscles of patients with oculobulbar myasthenia gravis was a reduction in the mean amplitude of the muscle action potential in response to a single nerve stimulus.

The partial block of transmission at the neuromuscular junction in generalized myasthenia gravis manifested several characteristic features:

1. There was a slight degree of block to the passage of a single impulse.

2. Following the passage of a single impulse there was an increased degree of block to the passage of a second impulse.

3. A train of impulses to the motor nerve resulted first in a progressive increase in block to the passage of the impulses across the neuromuscular junction. This was followed by a transient decrease in block, which in turn was followed by a progressive increase in degree of block. The magnitude of these changes increased as the stimulating frequency was raised.

4. Repetitive nerve stimulation was followed after a brief interval by a period of facilitation of neuromuscular transmission. This post-tetanic facilitation represented a decrease in the myasthenic block and was probably prejunctional in origin. The degree of post-tetanic facilitation was increased by increasing the duration or frequency of the tetanic stimulation.

The similarity between this neuromuscular block in myasthenia gravis and the block produced by partial curarization was pointed out. The possible implications of this similarity were discussed.

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STUDIES IN NEUROMUSCULAR FUNCTION

III. Stimulating and Depressant Effects of Acetylcholine and Choline in Normal Subjects[1, 2]

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The actions of acetylcholine (ACh) on the neuromuscular apparatus have been studied extensively in experimental animals. It was demonstrated by Brown (3) that this substance, when rapidly injected into a localized muscle area during temporary occlusion of the arterial blood supply, is capable of producing a muscle contraction, simulating that brought about by stimulation of the motor nerve. The resulting contraction is a brief asynchronous tetanus of the muscle fibers. The effect of ACh is to bring about depolarization of the motor end-plates (4, 5). There is no propagated muscle contraction following the application of ACh to other regions of the muscle fiber. When large doses of ACh are injected intra-arterially, the excitatory effect is followed by a paralytic effect, due to persistent depolarization of the motor end-plates which spreads to adjacent regions (6). As long as the end-plate region remains depolarized it cannot be stimulated through the motor nerve.

The gross muscle movements produced by the stimulating effect of small doses of ACh administered intra-arterially have been studied in human subjects (7–11). No systematic observations have been made concerning the blocking action of larger doses of ACh on neuromuscular transmission in man. The present study is concerned with the effect of intra-arterially injected ACh and choline on neuromuscular function in normal man. Particular attention has been paid to the concentrations necessary to cause stimulation of skeletal muscle and to produce block of neuromuscular transmission, as reflected by changes in the size of the action potential of the muscle in response to supramaximal motor nerve stimulation. The methods employed in this study were previously described (12).

ACh was found to produce transient stimulation of motor activity, followed by a brief period of depression of neuromuscular transmission. After recovery from this "prompt" depressant effect of ACh there was a "late" and more

[1] This work was supported by a contract between the U. S. Army Chemical Corps and the Johns Hopkins University, and was aided by grants from the Division of Neurologic Diseases and Blindness, National Institute of Health, U.S.P.H.S., and from the Smith, Kline and French Foundation.

[2] Part of this study has been presented in preliminary reports (1, 2).

prolonged reduction in function. The "prompt" depression was increased and prolonged by the prior administration of neostigmine, and thus appears to be due to persistence of the initial depolarization caused by the injected ACh. In contrast, the "late" depression was not increased, and therefore, does not appear to be a direct effect of ACh. The time course of the "late" depression, and its resemblance to a comparable degree of block produced by the injection of choline, suggested that it may be due to choline released as a result of hydrolysis of ACh.

RESULTS

Effects of acetylcholine

Stimulating effect of ACh

The intra-arterial injection of 0.1 to 2 mg. of ACh resulted in involuntary movement of the fingers in each of 35 normal subjects. There were usually several small flexion twitches of the fingers, with abduction of the fifth finger, and rippling movements of the hypothenar and thenar muscles. These began approximately 4 seconds after injection and recurred over a period of 5 to 15 seconds. The injection of 2 to 10 mg. produced more sustained flexion of the fingers and hand, accompanied in some instances by pronation. Larger doses (25 to 40 mg.) produced less visible movement, or, in some instances, none.

The threshold dose of ACh for eliciting involuntary movement varied in different subjects, the range being 0.1 to 2.0 mg. The mean in the 35 individuals studied was 0.65 mg., and the standard error of the mean 0.08 mg. It varied by as much as 0.4 mg. in the same subject when injections were administered at intervals of from ten minutes to several days. When the same dose was injected at intervals of less than ten minutes, the initial response was usually the most marked, so that the threshold for involuntary movement tended to be lower when it was determined by widely rather than closely spaced injections.

In most subjects, random muscle action potentials of 20 to 40 microvolts amplitude occurred spontaneously, in the absence of drug injection or nerve stimulation, probably due to slight muscle stretch. They could be recorded from concentric needle electrodes in the *m. abductor digiti quinti*. Two to five seconds after the intra-arterial injection of 0.2 mg. or more of ACh a burst of muscle action potentials varying from 50 to 100 microvolts appeared. These occurred in each of five subjects studied coincident with the gross movements described above, and lasted for 5 to 10 seconds. It is to be emphasized that during the period of these observations no stimuli were applied to the motor nerve.

"Prompt" depressant effect of ACh

Description and time course: Following the administration of 0.2 mg., or more, of ACh, and immediately after the brief stimulating effect described above, spontaneously occurring potentials disappeared for a period of about 20 seconds. During this time there was also reduction in amplitude of muscle action potentials induced by supramaximal nerve stimuli, in each of 25 normal subjects (Fig. 1). When a train of four to six stimuli 40 msec. apart was applied to the nerve, each of the muscle action potential responses was depressed to the same degree (Figs. 1 and 2). The time course of this effect of ACh was determined by applying these trains to the nerve every two seconds. The depression of muscle potential responses began in an average of five seconds, became maximal in seven seconds and disappeared in seventeen seconds.

When ACh was injected during a series of short trains of nerve stimuli, the pattern of depression of successive responses varied with the frequency of nerve stimulation. At frequencies of 50 per second or less each of the successive potentials was depressed to an equal degree during the period of maximal effect of injected drug (Figs. 1 and 2). At a frequency of 100 per second,

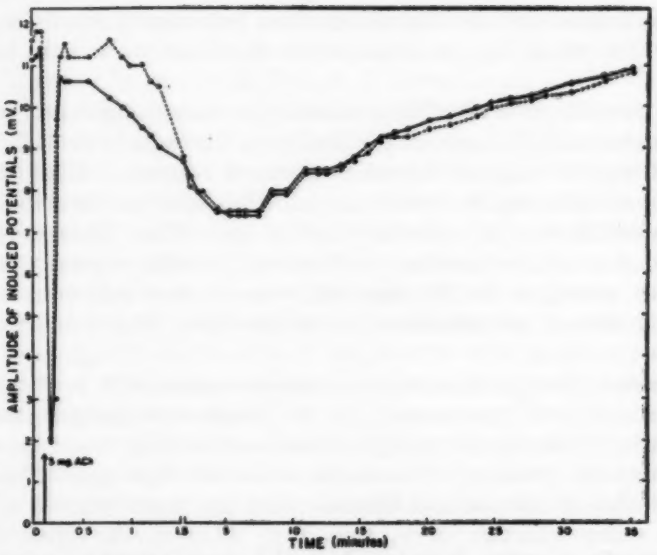

FIG. 1. Time course of the "prompt" and "late" depressant effects of intra-arterially injected ACh on the muscle action potentials in response to a train of four maximal nerve stimuli (40 msec. apart) evoked every 2 seconds. The amplitude (millivolts) of the responses to the first (●——●) and fourth (○– – –○) stimuli is plotted. (Normal subject, E. M.).

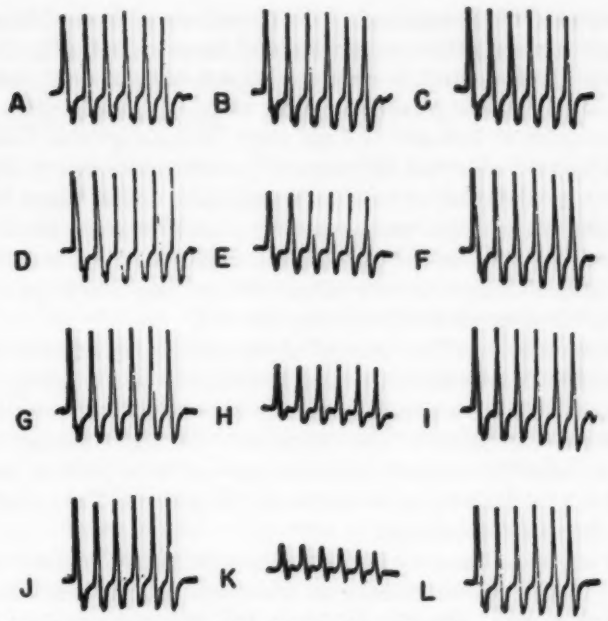

FIG. 2. "Prompt" depressant effect of increasing amounts of intra-arterially injected ACh on the muscle action potentials in response to a train of 6 nerve stimuli 40 msec. apart. First column (A, D, G, J): Control records, amplitude of initial potential 4.3 mV. Second column (B, E, H, K): Records taken 10 seconds after 0.6, 2, 5, and 10 mg. of ACh, respectively. Third column (C, F, I, L): Records taken 20 seconds after the injections of ACh. (Normal subject, E. M.).

successive potentials of a train were depressed by ACh in a progressively increasing manner. When the train consisted of 12 potentials, the reduction in voltage of the last potential was 25 per cent greater than that of the initial potential. When ACh was injected during a period of continuous nerve stimulation at frequencies of 1 through 100 per second the degree of depression of the action potentials followed the same time course as during intermittent short trains of nerve stimuli. At no time did ACh result in an increase in amplitude of potentials, even after these had been depressed as the result of long continued nerve stimulation at rates of 25 per second or more.

When the dose of ACh was greater than 4 mg., detectable weakness of voluntary contraction of the injected extremity was noted. Following 25 to 40 mg., the weakness was marked, and lasted up to 30 seconds.

Dose-effect relationship: The smallest dose of intra-arterial ACh that produced detectable depression of muscle action potentials evoked by motor nerve stimulation was 0.2 to 0.8 mg. The degree and duration of depression

were proportional to the amount of ACh injected, up to doses which produced about 60 per cent reduction in amplitude of the potentials (Fig. 2). In this dosage range there occurred a mean of 1.35 mV (20 per cent) reduction in amplitude of the induced potentials per mg. of ACh injected, with a standard error of the mean of 0.18 mV (2.3 per cent). Increasing doses then usually had less depressant effect per mg. of ACh, so that the curve relating dose of drug and degree of depression became exponential in shape. When the dose of ACh administered was corrected for the arm volume below the site of injection the range and standard error of the depressant effect of ACh increased slightly, indicating that differences in arm volume did not play a large part in determining differences in reactivity to injected ACh.

Effect of neostigmine: *The "prompt" depressant effect of ACh was intensified and prolonged by the prior administration of neostigmine* (0.3 to 1.5 mg.) (Fig. 3). There was, in addition, a change from even to progressive depression of successive potentials by the ACh. The depression of the first of a series of induced potentials increased from an average of 2.5 per cent to 62 per cent per 0.1 mg. of ACh, and of the fourth from 2.5 to nearly 100 per cent. The duration of the depression increased several fold.

Effect of atropine: The prior intramuscular administration of 0.6 to 1.2 mg. of atropine sulfate had no influence on the effects of ACh. The intra-arterial

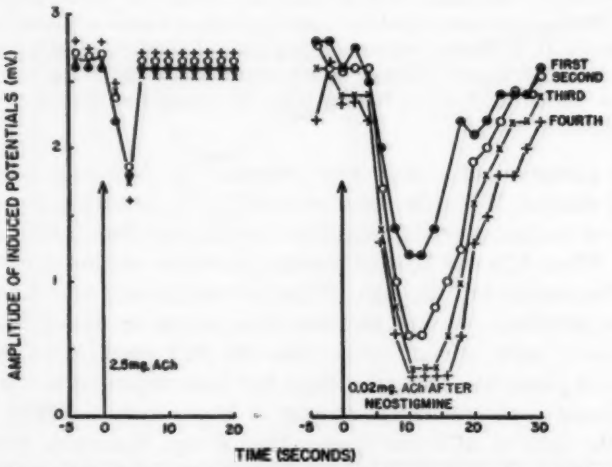

FIG. 3. Effect of neostigmine on the "prompt" depressant action of ACh on muscle action potentials evoked by nerve stimulation. Neostigmine (1.2 mg.) was administered intra-arterially 30 minutes before 0.02 mg. ACh, which had produced no change prior to neostigmine. (Normal subject, J. P.).

The prior administration of neostigmine intensified and prolonged the "prompt" depressant effect of ACh.

administration of one to three mg. of atropine sulfate had no influence on the neuromuscular effects or local pain produced by ACh, but did diminish the local vasodilatation and sweating.

"Late" depressant effect of ACh

Description and time course: After recovery from the transient "prompt" depression produced by a single dose of 1 to 10 mg. of ACh, there was a second, more prolonged and less marked reduction in amplitude of the muscle potentials evoked by nerve stimuli in each of 17 subjects (Fig. 1). This began an average of 1.2 minutes after injection, reached a maximum in 2.1 minutes and remained at this level for 10 minutes. The muscle action potentials then gradually returned to their initial amplitude over the next 30 to 60 minutes. The degree and duration of the "late" depression were roughly proportional to the dose of ACh, and were the same whether frequent test volleys were applied to the nerve (every 5 to 10 seconds), or whether sampling was performed at intervals of several minutes. The mean "late" depression was 0.52 mV (5.8 per cent) per mg. of ACh injected, with a standard error of the mean of 0.12 mV (1.3 per cent). Doses of ACh which were too small to produce any "prompt" depression had no "late" depressant effect.

When ACh was injected repeatedly the greatest degree of "late" depression generally occurred after the first or second injection (Figs. 4 and 5). After the potentials had been reduced by 20 to 30 per cent, subsequent injections

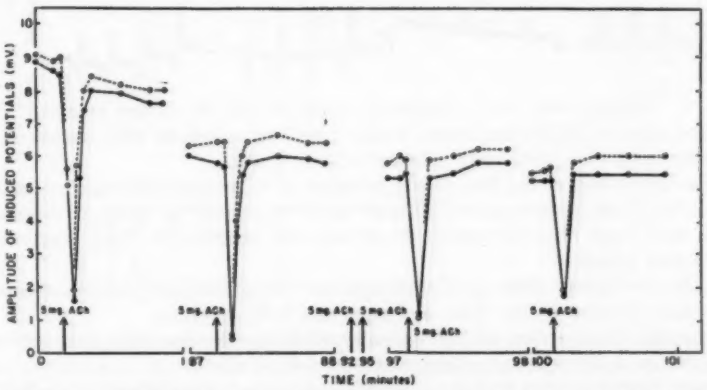

FIG. 4. "Prompt" and "late" depressant effects of successive intra-arterial injections of ACh on muscle action potentials evoked by nerve stimulation. The amplitude of the first (●——●) and fourth (○– – –○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every two seconds has been plotted. (Normal subject, T. P.)."

The 'late" depressant effect of ACh was most marked after the initial injection. It did not alter the "prompt" depressant effect of ACh, and was not reversed by ACh.

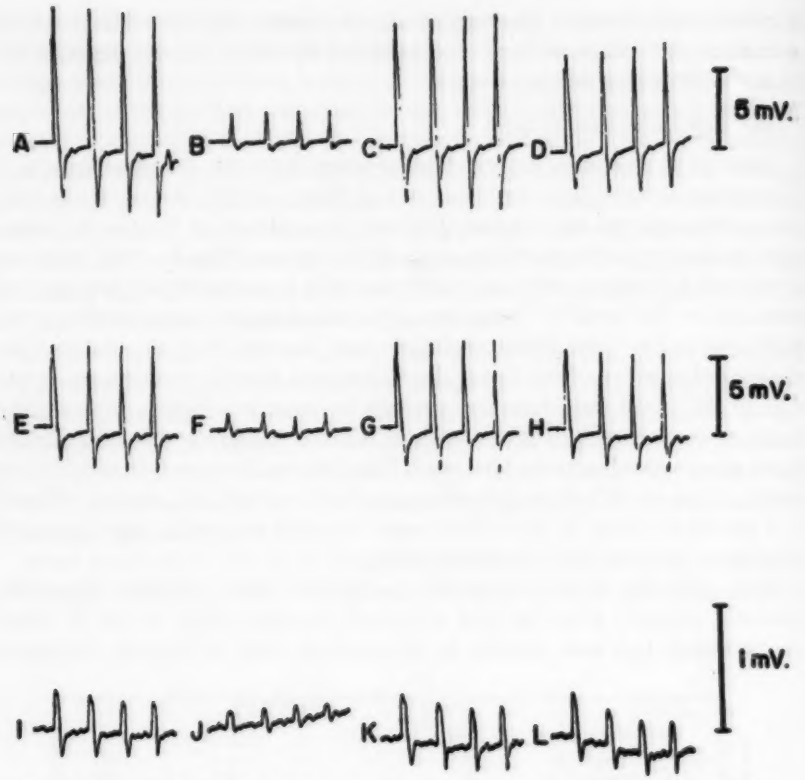

FIG. 5. "Prompt" and "late" depressant effects of ACh on evoked potentials (upper row), and effect of ACh administered during "late" depression by ACh (middle row) or depression by choline (lower row). (Normal subject, T. P.).

Upper row: Effect of the intra-arterial injection of ACh on muscle action potentials in response to a train of nerve stimuli 40 msec. apart. A, control. B, "prompt" depression 8 seconds after 5 mg. ACh. C, recovery 20 seconds after injection. D. "Late" depression 23 minutes after injection.

Middle row: Similar effect of ACh administered during the "late" depression produced by the prior injection of four doses of 5 mg. ACh. E–H, as above.

Lower row: Similar effect of ACh administered during the depression produced by the prior injection of 100 mg. choline intra-arterially. I–L, as above.

Neither "late" depression by ACh nor depression by choline appreciably altered the "prompt" depressant effect of ACh, or was reversed by ACh.

usually had little or no additive effect. Following repeated injections of ACh, totalling 14 to 51 (average 26) mg., the mean "late" depression was 2.2 mV (24 per cent). The successive responses to a train of nerve stimuli were equally depressed (Fig. 5), as were the responses to two stimuli delivered at intervals

greater than 16 msec. There was a slight increase in the response to a single nerve stimulus applied one to ten seconds after a train of 121 stimuli at 200 per second; i.e. there was slight post-tetanic facilitation.

Effect of ACh or neostigmine: *The "prompt" depressant effect of ACh or neostigmine was not altered during the "late" depression produced by ACh* (Figs. 4–6). The average "prompt" depression produced by 2 to 10 mg. of ACh in seven subjects was 78 per cent following the initial injection, and 68 per cent when the same dose was administered during the "late" depression. *Neither ACh* (1 to 20 mg.) *nor neostigmine* (0.1 to 2 mg.) *reversed the "late" depression produced by ACh;* i.e. they produced no increase in the amplitude of the evoked potentials.

The prior administration of neostigmine did not enhance or prolong the "late" depressant effect of ACh. In some instances this effect was diminished.

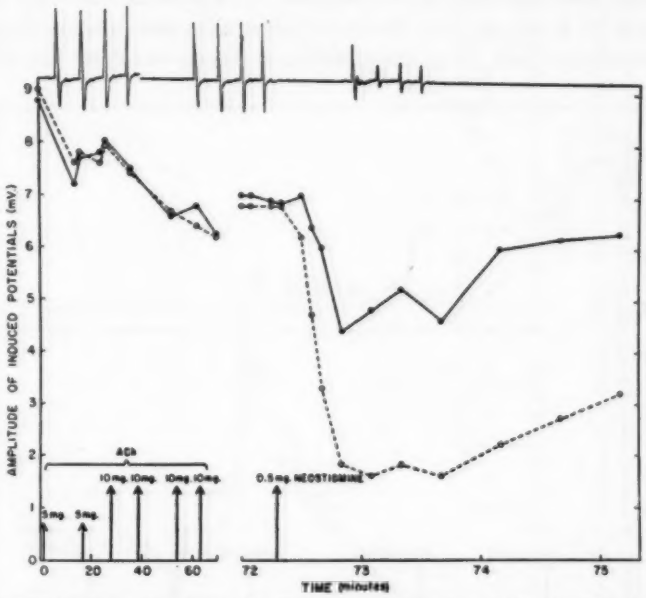

FIG. 6. Effect of neostigmine on the "late" depression of evoked potentials produced by ACh. The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○– – –○) of a train of nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial administration of ACh (showing only the "late" depressant effect) and neostigmine.

Above: left, control muscle action potentials; middle, "late" depression 12 minutes after last injection of ACh; right, progressive depression, and repetitive firing after the initial potential, one half minute after the injection of neostigmine. (Normal subject, E. N.).

The "late" depression produced by ACh did not alter the depressant effect of neostigmine, and was not reversed by this drug.

Depressant effect of choline

The rapid onset and brief duration of the "prompt" depressant effect of ACh, and its enhancement and prolongation by the prior administration of neostigmine, suggested that this effect is due to ACh itself. On the other hand, the slow onset and greater duration of the "late" depressant effect, and failure of the prior administration of neostigmine to enhance or prolong this effect, suggested that it may be due to one of the products of hydrolysis of ACh. The intra-arterial injection of sodium acetate, in doses up to 200 mg., had no effect. The administration of 5 to 30 mg. of choline chloride to 12 subjects resulted in depression of muscle action potentials evoked by nerve stimulation by a mean of 0.14 mV (2 per cent) per mg. of choline (Fig. 7). Doses of 50 to 100 mg. produced greater depression, but the dose-effect ratio decreased, approaching 0.07 mV (1 per cent) per mg. of choline. The effect began a mean of 8 seconds after injection and was maximal in 24 seconds. Recovery occurred slowly over 10 to 60 minutes. When the same dose was injected repeatedly, the initial injection had the greatest effect and subsequent doses had diminish-

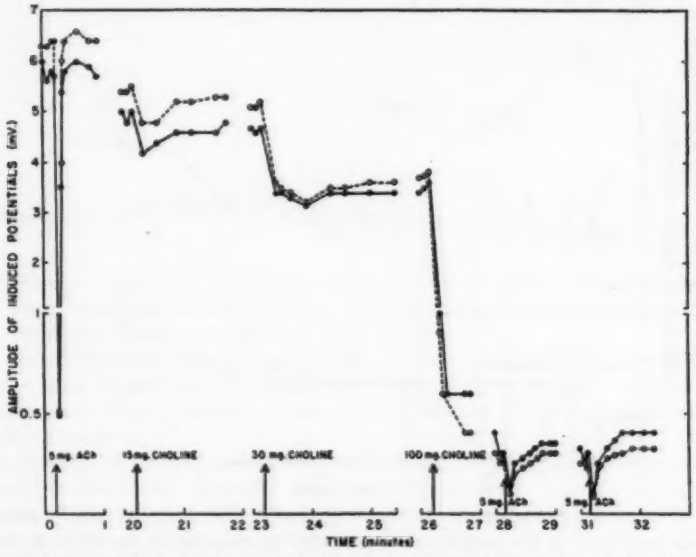

FIG. 7. Effect of ACh administered before and after depression of evoked potentials by choline. The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of nerve stimuli (40 msec. apart) evoked every 2 to 5 seconds is plotted before and after the intra-arterial administration of ACh, choline, and ACh after choline. (Normal subject, T. P.).

Marked choline block did not appreciably alter the "prompt" depressant effect of ACh, and was not reversed by ACh.

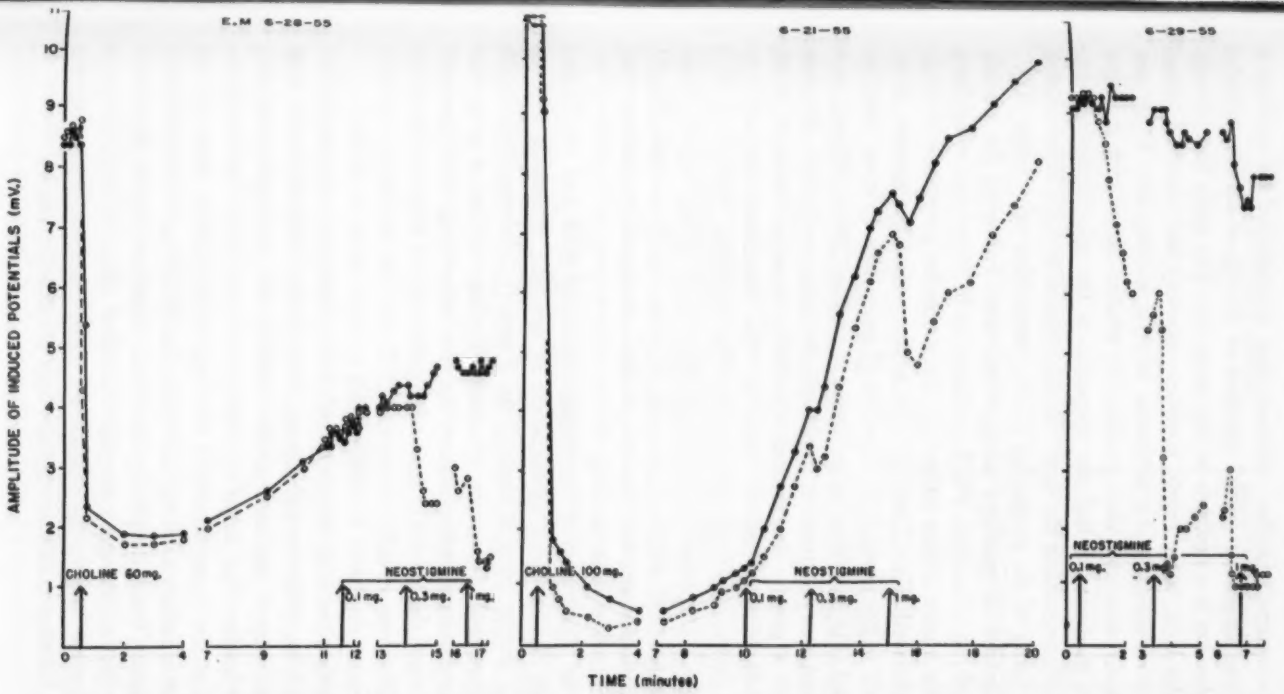

FIG. 8. Effect of neostigmine administered following depression of evoked potentials by choline. The amplitude of the muscle action potentials in response to the first (●——●) and fourth (○- - -○) of a train of nerve stimuli (40 msec. apart) evoked every 5 seconds is plotted before and after the intra-arterial administration of each of the drugs. (Normal subject, E. M.).

Left: *Moderate choline block produced even reduction of the potentials of each train, and was not reversed by neostigmine. The depressant effect of neostigmine was only slightly inhibited.*

Middle: *Marked choline block produced progressive reduction of the potentials of each train, and was reversed by neostigmine. The depressant effect of large doses of neostigmine was markedly inhibited.*

Right: Depressant effect of neostigmine alone.

ing effect. This occurred whether sufficient time was allowed for recovery or not. Thus, in one subject an initial injection of 20 mg. of choline produced 43 per cent reduction in amplitude of the potentials. The same dose administered 10 minutes later, after nearly complete recovery had occurred, caused only 28 per cent reduction, and a third dose 10 minutes later only 13 per cent.

The type of neuromuscular block produced by choline varied with the degree of block. When moderate, it had the properties of the non-competitive type (12, pages 118–19), resembling the "late" block produced by ACh. When marked, the block had many of the properties of the competitive type (page 118).

Effect on response to repetitive nerve stimulation: Following the administration of 5 to 60 mg. of choline, which produced up to 70 per cent reduction in amplitude of the muscle action potentials, the successive potentials of a train were evenly depressed (Figs. 7 and 8). The action potential responses to two stimuli delivered to the nerve at an interval of more than 16 msec. were also evenly depressed (Fig. 9). When larger doses of choline (100 to 500 mg.) were administered and more depression produced, the latter potentials of a train were reduced to a greater degree than the initial response, resulting in the appearance of decrement (Figs. 5, 7 and 8). This decrement frequently increased over a period of several minutes after the injection. There was also a greater reduction in the muscle potential response to the second of two stimuli delivered to the nerve at intervals between 16 msec. and 10 seconds (Fig. 9). At both moderate and marked degrees of block produced by choline, post-tetanic facilitation was observed (Fig. 9).

Effect of ACh: The "prompt" depressant effect of ACh was not altered during moderate choline block, as measured by per cent depression, but was slightly reduced during marked block (Figs. 5 and 7). The amplitude (millivolts) of the "prompt" depression was less following choline, in proportion to the reduction in amplitude produced by the latter drug. ACh produced approximately equal depression of successive potentials, so that any decrement caused by choline was usually preserved. ACh did not accelerate recovery from the depression produced by either small or large doses of choline.

Effect of neostigmine: *Moderate degrees of choline block were enhanced by small doses of neostigmine, while marked degrees of block were reversed by this drug.* When the block was of moderate degree, having the properties previously described, neostigmine, in doses of 0.3 mg. or more, produced further depression, especially of the latter potentials of a train (Fig. 8, left). However, the depressant effect of neostigmine was less than when this drug was administered alone (Fig. 8, right). When the choline block was of marked degree, small doses of neostigmine (0.1 to 0.3 mg.) reversed the block (Fig. 8, middle). Larger doses of neostigmine (e.g., 1 mg.) produced slight transient depression, but this was much less than when neostigmine was administered alone.

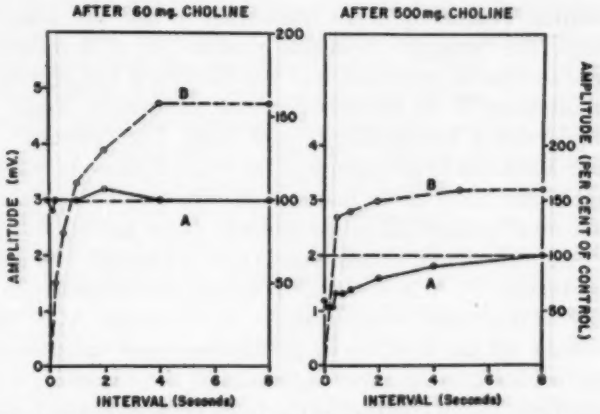

FIG. 9. Effect of choline on the muscle action potential response to repetitive nerve stimulation. Left: Following moderate depression by 60 mg. choline, intra-arterially. Right: Following marked depression by 500 mg. choline. (Normal subject, G. Y.).

A (●——●), amplitude of the muscle action potential response to the second of two nerve stimuli plotted against the time interval between the two stimuli, following depression by choline. The amplitude of the response to the first of the two stimuli is indicated by the broken line (100%). Prior to choline administration the amplitude of the potentials in response to the two stimuli was equal, and measured 10.2 mV. *Following moderate depression by choline there was equal reduction of potentials evoked by two nerve stimuli (left), while following marked depression, the second potential was reduced to a greater degree than the first (right).*

B (○– – –○), amplitude of the muscle action potential response to a single stimulus, delivered after a train of 121 repetitive stimuli applied at a rate of 200 per second, plotted against the time interval between the end of the train and the single stimulus, following depression by choline. The amplitude of the response to the first stimulus of the train is indicated by the broken line (100%). *Following both moderate and marked depression by choline there was post-tetanic facilitation;* i.e. there was an increase in the response to a single stimulus applied after a train.

DISCUSSION

The stimulating and "prompt" depressant effects of intra-arterially administered ACh on neuromuscular transmission have been previously observed in experimental animals (3–6). These effects have also been elicited in man, as manifested by the occurrence of flexion twitches, followed by transient weakness, in the injected extremity (7–11). Observations in experimental animals have indicated that the stimulating effect of ACh is due to the initiation of depolarization of the motor end-plates, and that the "prompt" depressant effect which immediately follows results from persistent depolarization of the end-plates and of the adjacent muscle fiber, rendering these areas incapable of responding to endogenous ACh liberated from the nerve endings (4–6). The studies described in this communication on the effect of ACh on muscle

action potentials evoked by nerve stimulation in man are compatible with these findings. The "prompt" depression produced by ACh resulted in equal depression of successive potentials, and was intensified and prolonged by the prior administration of an anticholinesterase compound. These properties are compatible with a depolarizing type of block. The "prompt" depressant effect of ACh is not due to persistence of the initial increase in motor activity, which might render many of the motor units refractory to the nerve impulse; in fact, the normal background of spontaneous motor activity was suppressed during the period of reduced responsiveness to nerve stimuli. Nor is it attributable to inactivation by ACh of sufficient muscle cholinesterase to cause the accumulation of depressant concentrations of endogenous ACh. Inactivation of cholinesterase by the injection of anticholinesterase compounds, such as neostigmine, results in progressive depression of the successive responses to nerve stimulation (13), in contrast to the equal depression which followed the injection of ACh.

The prior administration of neostigmine resulted not only in a marked increase in the degree and duration of the "prompt" depressant effect of ACh, but also in a change from even to progressive depression of the successive potentials of a train. The occurrence of progressive depression of evoked potentials is attributable to the accumulation of endogenous and exogenous ACh due to inhibition of cholinesterase.

The "late" depression observed following the administration of ACh has not been previously described in mammals. Thesleff (14) has reported that the depolarizing block produced by ACh in the frog was followed by a more prolonged block, during which the membrane potential was normal and the depolarizing action of ACh was inhibited. The block was not reversed by ACh. The "late" depression produced by ACh in man did not inhibit the "depolarizing" action of ACh, as indicated by the "prompt" depressant effect of this drug. It resulted in even depression of successive muscle potentials evoked by repetitive nerve stimuli, and was not reversible by ACh or neostigmine. It is, therefore, a non-competitive type of block. While this may be due to persistent depolarization of the region of the motor end-plates, such a mechanism cannot be established without actual measurement of the membrane potential.

Since this "late" blocking action of ACh was not increased by the prior administration of neostigmine, it seems clear that it is not a direct effect of ACh itself. The latent period of one to two minutes that occurred between the injection and the onset of the "late" depression suggests that it may be due instead to one of the products of hydrolysis of ACh. Intra-arterially injected sodium acetate had no effect on neuromuscular function, but choline produced prolonged depression. The properties of the block produced by choline varied with the degree of depression. *Moderate* degrees of block resembled in many ways the "late" depression proudced by ACh. Successive potentials of a train

evoked by repetitive nerve stimulation, and potentials evoked by paired stimuli at varying intervals, were evenly depressed. The administration of ACh or neostigmine intensified the neuromuscular block produced by choline, and the depressant effect of these drugs was only slightly less than prior to choline administration. Moderate depression due to choline was not reversed by ACh or neostigmine. The most striking difference between the depressant action of choline and the "late" depressant effect of ACh was in the dose-effect relationship, ACh producing three times as much depression per mg. of injected drug. Since each mg. of ACh yields on hydrolysis 0.8 mg. of choline, one might expect ACh to have slightly less effect than equal doses of choline. However, it is possible that ACh hydrolyzed at the neuromuscular junction by muscle cholinesterase may give rise to choline in closer proximity to the end-plates than would result from the injection of choline, since ACh may penetrate more readily into this region by virtue of its ionic interaction with the receptor protein. On the other hand, a degree of depression comparable to marked choline block could not be produced by the "late" depressant action of ACh, in the doses that were administered.

The only property of moderate choline depression, or of the "late" ACh depression, that suggested a competitive type of block was post-tetanic facilitation. This was more marked following choline than ACh. There is indirect evidence, based on measurement of end-plate potentials in the curarized animal, that post-tetanic decurarization is due to an increase in the output of endogenous ACh by the motor nerve endings following a tetanus (15, 16). In view of this evidence the finding of post-tetanic facilitation is generally regarded as evidence of the presence of a competitive type of block. Since there was no other evidence of this type of block following ACh or moderate doses of choline, it would appear that either post-tetanic facilitation is a more sensitive index of competitive block than is the response to repetitive nerve stimulation, or to ACh or neostigmine, or it may not always be attributable to an increase in the output of endogenous ACh by the motor nerve endings.

Marked depression of evoked muscle potentials by choline had many of the properties of a competitive type of block. These resembled the prototype competitive block produced by d-tubocurarine in that there was progressive depression of successive potentials of a train evoked by repetitive nerve stimulation, and of the second response to paired stimuli separated by varying intervals. There was reversal of the block by small doses of neostigmine, though not as striking as in the case of d-tubocurarine (17), and there was inhibition of the depressant effect of large doses of neostigmine. Marked choline block differed from that produced by d-tubocurarine in that there was only slight inhibition of the "prompt" (depolarizing) action of ACh, and the block was not reversed by ACh.

In the cat, the intravenous or intra-arterial administration of choline was found to produce muscular fasciculations followed by neuromuscular block (in the anterior tibial muscle) attributable to persistent depolarization of the motor end-plate region (15). There was no evidence that choline, even in large doses, had a competitive blocking action. Repetitive stimulation of the nerve to the anterior tibial muscle did not restore transmission that had been blocked by choline; i.e., there was no post-tetanic facilitation, in contrast to the findings that have been presented in man. The action of choline on the anterior tibial muscle of the cat resembled that of decamethonium (6), except that choline produced not only depolarization of the end-plate region, but, in addition, an increase in the end-plate potential. This finding led to the suggestion that, in this species, choline may increase the output of ACh from the motor nerve endings (15), as it is reported to do in sympathetic ganglia (20). While choline causes slight inhibition of cholinesterase in vitro (21), it did not alter the time course of the end-plate potentials recorded in the cat (15), indicating that the neuromuscular effects were not attributable to cholinesterase inhibition.

In man, the response of *m. abductor digiti quinti* to choline resembles the response to decamethonium, moderate degrees of block being of the non-competitive type, and marked block of the competitive type, except for only slight inhibition of the "prompt" (depolarizing) action of ACh (22). It is of interest that, in the cat, the block produced by decamethonium in the tibialis, a pale muscle, is of the non-competitive type, while in the soleus, a red muscle, it is initially non-competitive and then competitive (23). Most human muscles, including *m. abductor digiti quinti*, contain a mixture of pale and red fibers.

The possible role of the depressant effect of endogenous ACh and choline in various disorders of neuromuscular transmission, and in the phenomenon of fatigue in normal individuals, will be the subject of subsequent communications.

SUMMARY

The intra-arterial injection of acetylcholine (ACh) in normal subjects produced the following results:

1. Transient stimulation of motor activity attributable to depolarization of motor end-plates.

2. "Prompt" transient depression of neuromuscular transmission which appeared to be due to persistence of the initial depolarization, rendering the end-plates insusceptible to stimulation by endogenous ACh.

3. "Late" depression of neuromuscular transmission which had the characteristics of a non-competitive type of block (even depression of successive potentials in a train and enhancement of the block by ACh or neostigmine). This block did not appear to be due to ACh itself. Its time course, and re-

semblance to a comparable degree of block produced by the intra-arterial injection of choline, suggested that it may be due to choline released as a result of hydrolysis of ACh. While a moderate degree of block produced by choline was of the non-competitive type, marked block had many of the properties of the competitive type, including progressive depression of successive potentials in a train and reversibility by neostigmine.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Victor A. McKusick for his help in the early phases of this study. George I. Johnston, Leroy H. Warthen, Olive L. Smith and Madeleine Alonso-Lej gave valuable technical assistance.

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STUDIES IN NEUROMUSCULAR FUNCTION

IV. Stimulating and Depressant Effects of Acetylcholine and Choline in Patients with Myasthenia Gravis, and Their Relationship to the Defect in Neuromuscular Transmission[1, 2]

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A single supramaximal stimulus delivered to a motor nerve in patients with myasthenia gravis produces a muscle action potential and twitch that are below the normal range in amplitude only when there is severe weakness of the muscle tested (3). However, in all myasthenic patients, repetitive stimulation of the nerve to affected muscles results in progressive decline of the responses, at frequencies which produce responses of even amplitude in normal subjects. The degree and rate of decline are roughly proportional to the frequency of nerve stimulation and the degree of involvement of the muscle tested. These manifestations are believed to be due to impairment of neuromuscular transmission. The abnormal fatigue and the progressive decline of the responses to nerve stimulation indicate that neuromuscular activity, whether voluntary or induced, increases the degree of this impairment.

The administration of anticholinesterase compounds, such as neostigmine, results in improvement in the weakness and abnormal fatigability (4). This indicates that the defect is probably due to deficient action of transmitter substance (acetylcholine, ACh) on the motor end-plate. This could be due to deficient release of transmitter substance from the motor nerve endings, to its excessively rapid removal, or to interference with its depolarizing action on the end-plate. The effect of neostigmine does not enable these possibilities to be differentiated. The synthesis of ACh by motor nerve endings cannot be studied directly. The plasma of patients with myasthenia gravis has been reported to inhibit the synthesis of ACh by extracts or homogenates of brain tissue (5, 6), but recent studies have failed to disclose any difference in the effect on this synthesis of plasma or muscle homogenates obtained from normal and myasthenic subjects (7). No difference has been found in the cholinesterase activity of homogenates of muscle obtained from normal and myasthenic subjects

[1] This work was supported by a contract between the U. S. Army Chemical Corps and The Johns Hopkins University, and was aided by grants from the Division of Neurologic Diseases and Blindness, National Institute of Health, U.S.P.H.S., and the Myasthenia Gravis Foundation.

[2] Part of this study has been presented in preliminary reports (1, 2).

(8–10), but comparative studies on the distribution and concentration of the enzyme in the region of the end-plates have not been reported. Numerous observations have pointed to similarities between the neuromuscular defect in myasthenia gravis and that produced by the administration of d-tubocurarine to normal subjects or experimental animals (4, 11). These similarities suggest that the neuromuscular block in myasthenia is of the competitive type.

In an effort to gain further information concerning the nature of the defect in neuromuscular function in myasthenia, the effects of intra-arterially administered ACh and choline have been studied. The methods employed were previously described (11).

As in normal subjects (12), intra-arterially injected ACh produced transient stimulation of motor activity, a "prompt" transient depression of neuromuscular transmission, and a "late" and more prolonged depression. There was also interposed a transient improvement in neuromuscular function. Again, as in normal subjects, the "prompt" depression was increased and prolonged by the prior administration of neostigmine. On the other hand the "late" depression was reversed by neostigmine. Thus, in myasthenia the "late" depression has the properties of an ACh-inhibitory, competitive block, in contrast to the non-ACh-inhibitory, non-competitive type that occurred in normal subjects (12). The relationship of ACh and of choline released from hydrolysis of ACh to the production of the competitive block in this disease will be discussed.

RESULTS IN PATIENTS WITH GENERALIZED MYASTHENIA GRAVIS

Effects of Acetylcholine

Stimulating Effect of ACh

Within 2 to 5 seconds after the injection of 0.03 to 5 mg. of ACh into the brachial artery there was a burst of action potentials (recorded from concentric needle electrodes in the *m. abductor digiti quinti*), accompanied by involuntary flexion twitches of the fingers and rippling movements of the hypothenar and thenar muscles. These effects were similar to those observed in normal subjects. The mean threshold concentration of ACh required to elicit a visible twitch was twice that in normal subjects (Fig. 1). However, the range was so wide in each group that this difference is barely significant ($p < 0.05$). The threshold concentration was not significantly different in patients with extremely weak arm and hand muscles than in those with mild weakness. One patient with severe generalized myasthenia responded to as little as 0.03 mg. The degree and duration of involuntary movement were in general the same as in normal subjects, although ACh elicited more marked and more sustained flexion of the fingers and supination of the hand in two patients than in any of the normal subjects. The time course of the potentials produced was the same as in normal subjects.

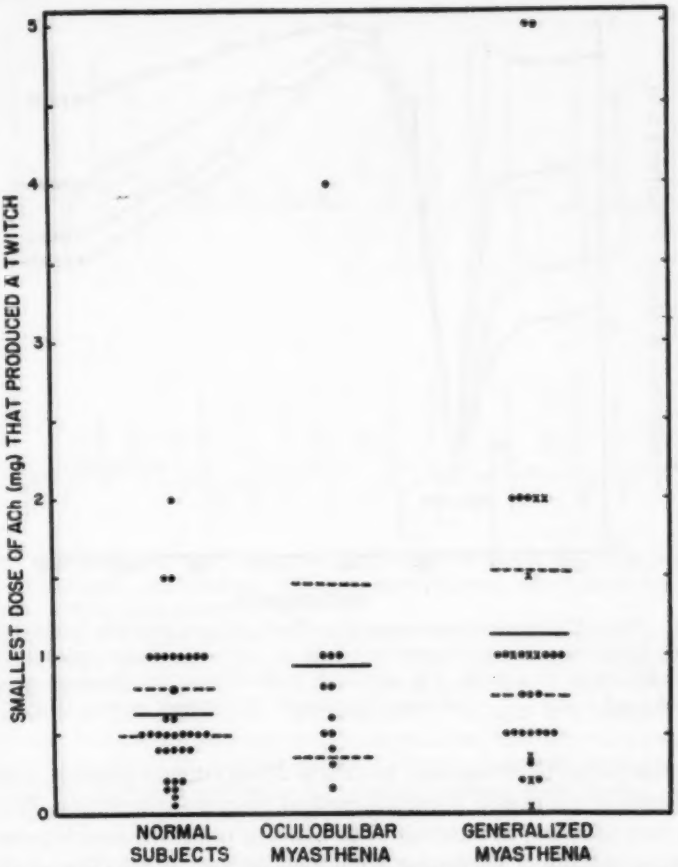

FIG. 1. Comparison of the stimulating effect of ACh in normal subjects and in patients with oculobulbar and generalized myasthenia gravis, as indicated by the smallest dose of intra-arterially injected ACh that produced a visible twitch in the hand muscles. The solid line indicates the mean for each group, and the dotted lines ± twice the standard error of the mean. Myasthenic patients whose grip strength was less than 8 kg. are indicated by an x. The only difference between the means which may be significant is that between normal subjects and patients with generalized myasthenia ($p < 0.05$).

"Prompt" Depressant Effect of ACh

Description and time course: In myasthenics, as in normal subjects, random muscle action potentials of 20 to 40 microvolts amplitude occurred spontaneously, probably as a result of slight muscle stretch. As in normal subjects, the motor activity produced by the administration of 0.2 mg., or more, of ACh was followed by suppression of these spontaneous potentials for about 20 seconds.

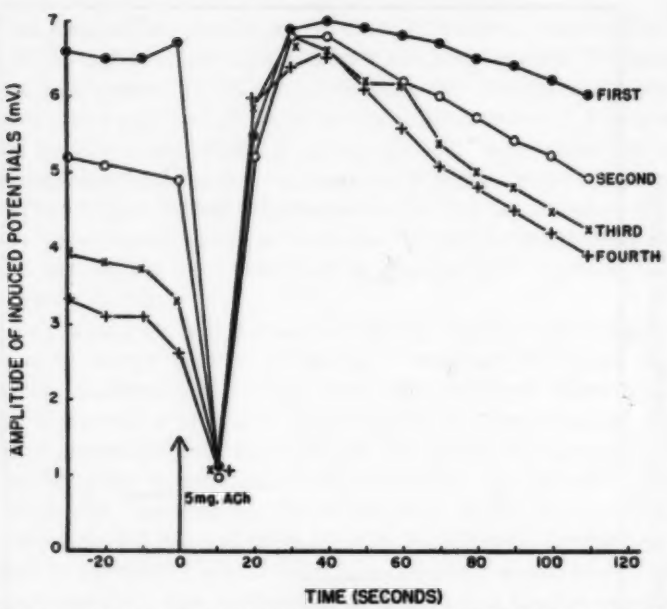

FIG. 2. "Prompt" depressant and reparative effects of intra-arterially injected ACh on the muscle action potentials in response to a train of four maximal nerve stimuli (40 msec. apart) evoked every 10 seconds. The amplitude (millivolts) of the responses to the first (●——●) and fourth (○- - -○) stimuli is plotted. (Myasthenic patient, R. P.).

During this period there was also, in each of 30 myasthenic patients, reduction in amplitude of the muscle potentials evoked by nerve stimulation. When the stimuli were delivered at intervals of 40 msec. or more, the muscle potentials, which were initially of decreasing amplitude, became equal (Figs. 2–4). The depressant effect of ACh on successive responses was, therefore, a diminishing one, in contrast to the even depression that occurred in normal subjects. The time course of the "prompt" depressant effect was the same as in normal subjects (Table I). As in normal subjects, the smallest dose of ACh which produced detectable depression of evoked potentials was slightly greater than that required to elicit involuntary movement; and with larger doses (5 to 20 mg.) the involuntary movement was followed by local weakness of the same degree and duration.

Effect on the initial potential of a train: The average "prompt" depressant effect of ACh on the first of a series of potentials evoked by nerve stimulation was less in patients with myasthenia than in normal subjects, but the ranges were so wide that the difference was not clearly significant, even in patients with severe weakness (Table II).

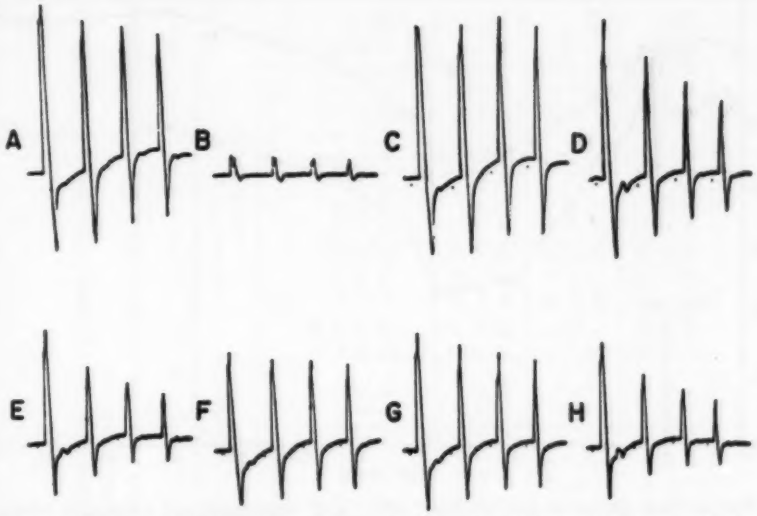

FIG. 3. "Prompt" and "late" depressant, and reparative effects of ACh on evoked po tentials (upper row), and effect of ACh administered during "late" depression by ACh (lower row). (Myasthenic patient, P. B.).

Upper row: Effect of the intra-arterial injection of ACh on muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 10 seconds. A, control (initial potential 6.8 mV.). B, "prompt" depression 10 seconds after 5 mg. ACh. C repair 25 seconds after injection. D, "late" depression 7 minutes after injection.

Lower row: Effect of the same dose of ACh injected after "late" depression produced by a total of 45 mg. ACh. E–H, as above, but there is now no "prompt" depression.

During "late" depression produced by ACh there was inhibition of the "prompt" depressant effect of ACh, and increase in the reparative effect of ACh.

Effect on the second and subsequent potentials of a train: *The "prompt" depressant effect on the second and subsequent muscle action potentials was significantly less than in normal subjects.* The mean amplitude of reduction of the fourth potential was one-third that in normals, and the mean per cent reduction one half (Table II). These differences are significant ($p < 0.01$). In patients with the most severe weakness, as much as 10 mg. of ACh had virtually no "prompt" depressant effect on the fourth potential of a train (Table II and Figs. 5 and 6). In two of these patients, when ACh was readministered several months later, following spontaneous increase in strength and in the amplitude of the fourth potential of a train, there was a corresponding increase in the "prompt" depressant effect. In general, the lower the amplitude of the latter potentials of a train, the less was the "prompt" depressant effect of ACh on these potentials.

Effect of neostigmine: *The "prompt" depressant effect of ACh was intensified*

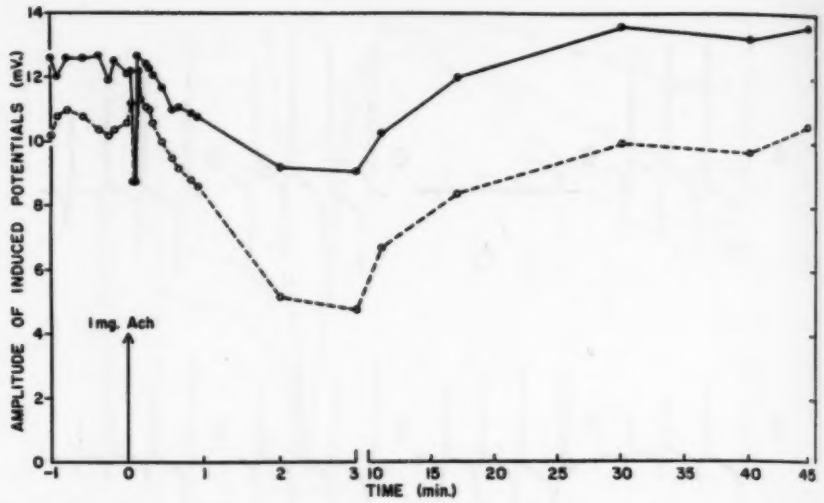

FIG. 4. Time course of the "prompt" and "late" depressant and reparative effects of intra-arterially injected ACh on the muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds. The amplitude of the responses to the first (●——●) and fourth (○– – –○) stimuli is plotted. (Myasthenic patient, J. G.).

TABLE I

Time Course of the "Prompt" and "Late" Depressant Effects of ACh, and of the Depressant Effect of Choline, in Normal Subjects and in Patients with Oculobulbar and Generalized Myasthenia Gravis (m.g.).

Compound	Dose (mg., mean)	Subjects	No.	Time (Seconds) Between Injection and Depression of Potentials Evoked by Nerve Stimuli	
				Onset ± S.E.	Maximum ± S.E.
ACh, "Prompt" Depressant Effect	5	Normal	25	5 ± 0.3	7 ± 0.3
		Oculobulbar m.g.	14	6 ± 0.5	8.5 ± 0.5
		Generalized m.g.	30	6.5 ± 0.3	9 ± 0.3
ACh, "Late" Depressant Effect	5	Normal	17	72 ± 5.4	126 ± 13.8
		Oculobulbar m.g.	11	72 ± 4.2	126 ± 13.2
		Generalized m.g.	23	66 ± 4.2	120 ± 12.0
Choline, Depressant Effect	45	Normal	17	7.5 ± 0.6	24 ± 2.6
		Oculobulbar m.g.	14	12 ± 1.0	40 ± 4.5
		Generalized m.g.	23	23 ± 1.7	104 ± 10.5
without preceding repair		Generalized m.g.	11	15 ± 0.9	99 ± 16.0
with preceding repair		Generalized m.g.	12	30 ± 2.3	109 ± 13.5

TABLE II

Comparison of the Depressant Effect of ACh and Choline on Muscle Action Potentials Evoked by Nerve Stimulation in Normal Subjects, Patients with Oculobulbar Myasthenia, and Patients with Generalized Myasthenia

The mean amplitude and per cent depression ± the standard error of the mean are recorded. P compares the patients with oculobulbar and generalized myasthenia with the normal subjects. Differences are not significant unless $P < 0.05$.

Compound and Dose (mg., mean)	Effect	Subjects	No.	Depression of Muscle Action Potentials Evoked by Nerve Stimuli at 40 msec. Intervals							
				First Potential				Fourth Potential			
				mV.	P<	Per Cent	P<	mV.	P<	Per Cent	P<
ACh, single dose (4 mg.)	"Prompt" depression per mg. ACh	Normal	20	1.35 ± 0.18		19.9 ± 2.3		1.35 ± 0.18		19.9 ± 2.3	
		Ocul. m.g.	12	0.95 ± 0.16	0.2	14.8 ± 2.4	0.2	0.95 ± 0.16	0.2	14.8 ± 2.4	0.2
		Gen. m.g.	26	0.99 ± 0.19	0.2	17.9 ± 2.9	0.6	0.47 ± 0.09	0.01	11.1 ± 2.2	0.01
		Gen. m.g., grip < 10 kg.	6	0.68 ± 0.37	0.2	13.9 ± 7.2	0.4	0.02 ± 0.01	0.001	1.2 ± 0.7	0.001
ACh, single dose (4 mg.)	"Late" depression per mg. ACh	Normal	18	0.29 ± 0.07		3.9 ± 0.8		0.31 ± 0.08		3.6 ± 0.9	
		Ocul. m.g.	18	0.15 ± 0.05	0.2	2.4 ± 0.7	0.2	0.16 ± 0.05	0.2	2.4 ± 0.7	0.2
		Gen. m.g.	28	0.50 ± 0.16	0.1	7.7 ± 1.3	0.02	0.81 ± 0.24	0.1	13.2 ± 2.3	0.001
ACh, repeated doses (20 mg. total)	"Late" depression, maximum	Normal	16	1.9 ± 0.36		22.0 ± 3.0		2.0 ± 0.45		21.1 ± 3.7	
		Ocul. m.g.	9	1.3 ± 0.29	0.3	16.7 ± 3.6	0.3	1.6 ± 0.45	0.5	18.3 ± 5.0	0.7
		Gen. m.g.	23	3.0 ± 0.60	0.2	44.0 ± 5.1	0.001	2.8 ± 0.55	0.3	59.0 ± 5.0	0.001
Choline, single dose (20 mg.)	Depression per mg. choline	Normal	15	0.14 ± 0.02		2.2 ± 0.21		0.16 ± 0.02		2.5 ± 0.2	
		Ocul. m.g.	16	0.09 ± 0.02	0.05	1.1 ± 0.17	0.01	0.11 ± 0.02	0.05	1.5 ± 0.2	0.01
		Gen. m.g.	16	0.11 ± 0.02	0.2	2.3 ± 0.41	0.9	0.24 ± 0.08	0.4	6.0 ± 0.5	0.001

FIG. 5. Effect of successive intra-arterial injections of ACh at 15 minute intervals on muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds in a patient with very severe myasthenia gravis (H. W.). "Prompt" depression occurred only in the initial potential and following the first injection of ACh. Partial repair occurred after each injection, but the degree of repair diminished, probably owing to the "late" depressant effect of ACh and the severity of the myasthenic defect.

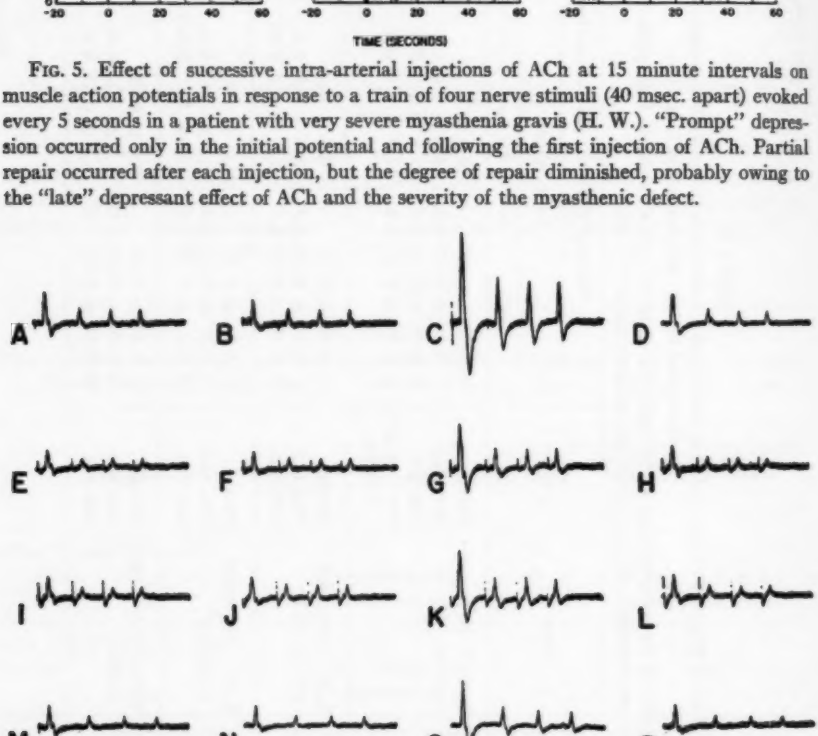

FIG. 6. Same legend as Fig. 5.

Upper row: A, control (initial potential 0.44 mV). B, "prompt" depression of initial potential 10 seconds after the injection of 5 mg. ACh. C, partial repair 15 seconds after injection. D, "late" depression 30 seconds after injection.

Second–Fourth rows: Effect of the injection of 20, 40, and 60 mg. ACh. E–P, as above.

and prolonged by the prior administration of neostigmine (0.3 to 2 mg.) (Figs. 8 and 9). There was, in addition, a change from even to progressive depression of successive potentials by the ACh.

Potentiating Effect of ACh

Following the "prompt" depression produced by the administration of 0.1 to 20 mg. of ACh, there was an increase in the amplitude of muscle action potentials evoked by nerve stimulation in each of 26 patients (Figs. 2–4). The degree varied roughly with the dose, up to approximately 10 mg., while larger doses had little additional effect (Figs. 5 and 6). The increase was most marked in those potentials of a short train which manifested the greatest degree of block. Thus, it was generally slight in the initial potential, and successive potentials became more nearly equal in amplitude. The degree of repair was proportional to the degree of involvement of the muscle tested by myasthenia. In patients with slight neuromuscular block, manifested by little reduction in amplitude of the second, third and fourth potentials, ACh produced an increase in their amplitude by 10 to 25 per cent. In patients with severe neuromuscular block, with marked reduction in all four potentials, ACh produced a marked increase, up to 300 per cent (Figs. 5 and 6). In 13 of 15 patients the amplitude of the potentials was increased to or near the normal range. In the two patients with the most severe myasthenia, who were unable to move their extremities when in the basal state, ACh produced a three fold increase in the voltage of all four potentials, but these remained considerably below the normal range (Figs. 5 and 6). In one patient, the administration of as much as 60 mg. ACh did not produce any further increase.

Following the injection of small doses of ACh (0.1 to 0.4 mg.) repair occurred without preceding depression. Larger doses of ACh (0.5 to 2 mg.) transiently reduced the response to the first, and sometimes the second stimulus before repair occurred, while the responses to the third and fourth stimuli were usually repaired without prior depression. Still larger doses of ACh (2.5 to 20 mg.) resulted, in all except the most severely myasthenic patients, in depression of all the potentials for approximately ten seconds prior to their repair.

The repair was maximal about 20 seconds after the injection of ACh (Fig. 2). The potentials then returned to their original amplitude over a period of about 140 seconds, with the fourth potential requiring the longest time to return. At certain stages during the development of the repair the second, third and at times even the fourth potential attained greater amplitude than the initial potential, i.e. facilitation appeared. With longer trains (16 stimuli), the increase in amplitude was generally greatest in the fourth potential, and less marked in subsequent ones.

The period of depression produced by ACh was of the same duration in myasthenic as in normal subjects. The period of depression plus repair in the

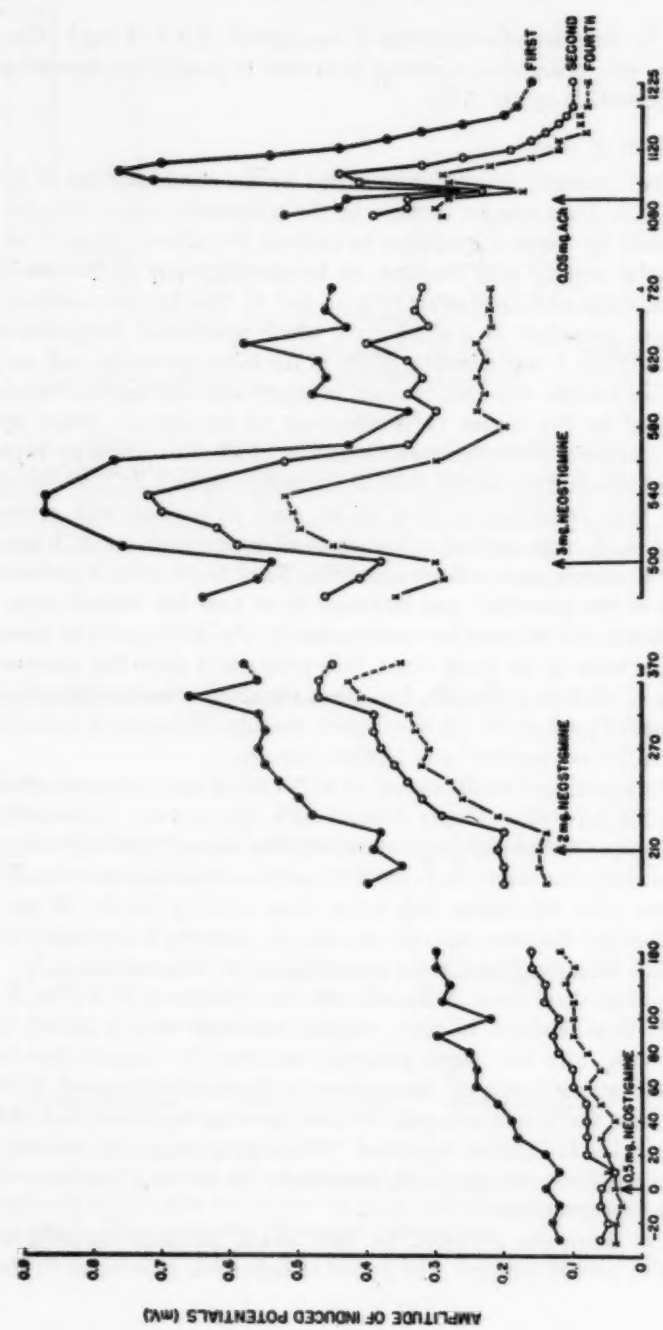

FIG. 7. Effect of successive intra-arterial injections of neostigmine on muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 10 seconds in a patient with very severe myasthenia gravis (H. W.). The first two injections produced partial repair, and the third, partial repair followed by depression. The subsequent intra-arterial injection of a small dose of ACh, which had no effect prior to neostigmine, produced transient depression, followed by repair, and then by more prolonged depression.

former was three times longer than the period of depression in the normal subjects (50 seconds).

Reparative effect of ACh administered during prolonged repetitive nerve stimulation: The injection of 2 to 10 mg. of ACh during prolonged repetitive nerve stimulation at rates of 10 to 50 per second, at a time when the muscle responses were moderately reduced, resulted in a 20 to 50 per cent increase in their amplitude. Following 5 mg. or more of ACh this potentiation was usually preceded by transient depression. When the amplitude of the action potentials was reduced by prolonged repetitive nerve stimulation to or near zero, these same doses had no observable effect.

Comparison between the reparative effect of ACh and neostigmine: The maximal reparative effect of ACh was equal to, or slightly less than that of neostigmine, but was more transient. In patients with a severe degree of myasthenic block, large doses of either ACh or neostigmine produced only partial repair of the evoked potentials (Figs. 7 and 8). The repeated administration of neostigmine resulted first in partial repair, and then in progressive depression of the potentials, without their first attaining equal amplitude, and while they were still far below the normal range of amplitude.

Effect of the prior administration of neostigmine: This resulted in reparative action by smaller doses of ACh than had previously exerted any effect (e.g. 0.05 mg., Fig. 7).

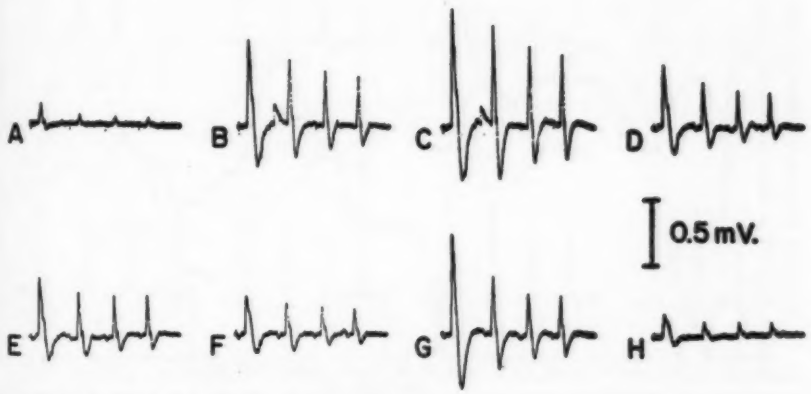

FIG. 8. Same legend as Fig. 7.

Upper row: A, control muscle action potentials in response to four nerve stimuli 40 msec. apart. B, partial repair after the intra-arterial injection of 0.5 and 2.5 mg. neostigmine. C, further repair after 3 mg. neostigmine, followed by D, depression.

Lower row: E, slight recovery. F, depression 5 seconds after the intraarterial injection of 0.05 mg. ACh, and 10 minutes after 3 mg. neostigmine. G, partial repair 15 seconds after the ACh. H, more marked depression 135 seconds after the ACh.

prior to neostigmine, produced transient depression, followed by repair, and then by more prolonged depression.

"Late" Depressant Effect of ACh

Following a single injection: The "prompt" depressant and reparative effects of ACh were followed by a prolonged period of depression of muscle action potentials evoked by nerve stimulation in each of 13 patients (Figs. 3 and 4). The degree of the "late" depression was roughly proportional to the dose of ACh injected over the range of 1 to 10 mg. Each of the successive potentials of a train was equally reduced, in terms of amplitude (millivolts), preserving the myasthenic decrement. Since the latter potentials of the train were lower than the initial potential prior to drug administration, the per cent reduction of these potentials was greater.

The "late" depression was more marked in the majority of myasthenic patients than in normal subjects, and was most marked in those patients with the severest weakness. When expressed as amplitude (millivolts) of depression, the difference between myasthenic patients and normal subjects was not significant, because of the wide range in both groups. However, when expressed as per cent depression, the difference was significant (Table II), owing to the lower amplitude of the potentials prior to drug administration in myasthenics than in normals.

The time between injection and "late" depression was the same as in normal

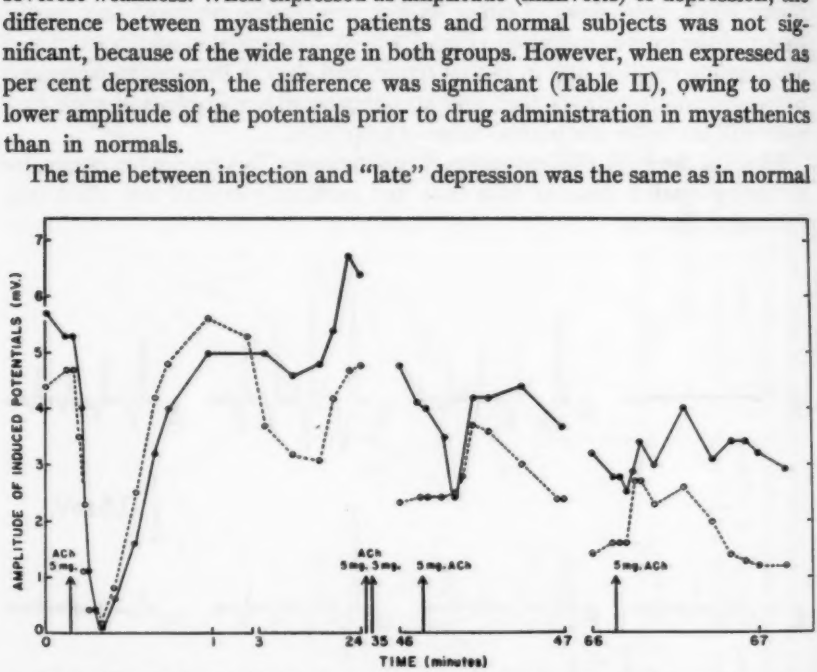

FIG. 9. "Prompt" and "late" depressant, and reparative effects of successive intra-arterial injections of ACh on muscle action potentials evoked by nerve stimulation. The amplitude of the first (●——●) and fourth (○- - -○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds has been plotted. (Myasthenic patient, R. A.).

Repeated injections of ACh produced increasing "late" depression, during which there was inhibition of the "prompt" depressant effect of ACh, and increase in the reparative effect of ACh.

subjects (Table I), but the duration of the depression was nearly twice as great.

Following repeated injections: Repeated injections of ACh had an additive effect, with increasing "late" depression (Figs. 9 and 10). This was more marked than in normal subjects (Table II).

Effect on response to repetitive nerve stimulation: The "late" depressant effect of ACh occurred whether the muscle was allowed to rest or was stimulated periodically through the nerve. When nerve stimulation was carried out, there was more marked decline (decrement) in the amplitude of successive muscle potentials than prior to ACh administration (Fig. 10). No such change was observed during simultaneous stimulation of the opposite uninjected extremity.

When a single or conditioning stimulus was delivered to the nerve and then a second or test stimulus applied at intervals from 16 msec. to 10 seconds, the decrease in the muscle action potential response to the second stimulus was more marked during "late" ACh depression (Fig. 11). When a single test stimulus was applied 0.5 to 10 seconds after a train of 121 stimuli delivered at a rate of 200 per second, the increase in the muscle action potential response to this test stimulus (i.e. the post-tetanic facilitation) was greater (Fig. 11).

Effect on "prompt" depressant action of ACh: *The "prompt" depressant ac-*

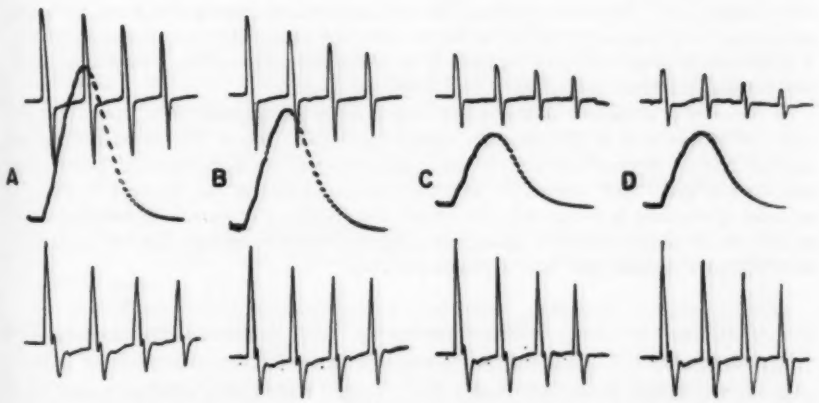

FIG. 10. Effect of successive intra-arterial injections of ACh on muscle action potentials (upper row) and twitch tension (middle row) evoked by nerve stimulation. A, control response to a train of four nerve stimuli 40 msec. apart (amplitude of initial potential 4.5 mV and of twitch tension (isometric) 1.5 kg.). B, five minutes after 2 mg. ACh. C, following five additional injections of 5 mg. ACh during one half hour after C. The lower row records muscle action potentials from the opposite (uninjected) extremity evoked by nerve stimulation concomitant with that which evoked the potentials in the upper row. (Myasthenic patient R. A.).

Successive injections of ACh produced increasing "late" depression.

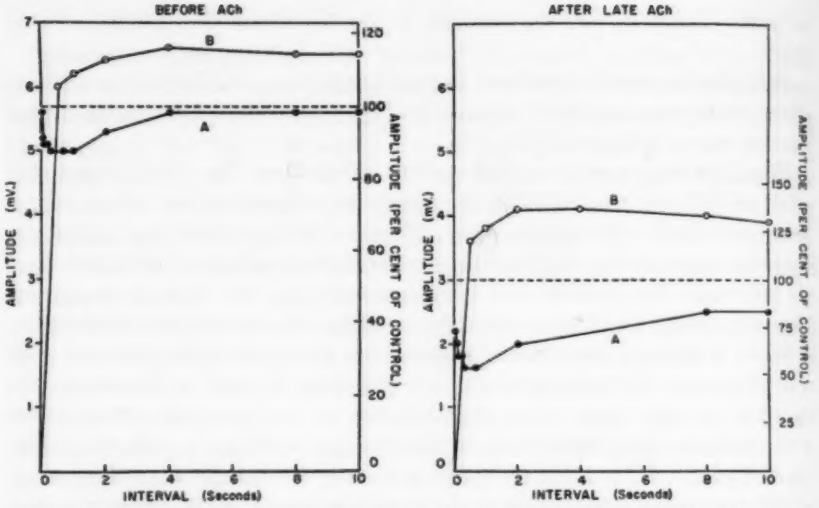

FIG. 11. Changes in the response of the muscle to nerve stimulation during the "late" depression produced by ACh. (Myasthenic patient, R. A.).

A (●——●), amplitude of the muscle action potential response to the second of two nerve stimuli plotted against the time interval between the two stimuli, before (left) and after (right) "late" depression produced by four intra-arterial injections of 5 mg. ACh. The amplitude of the response to the first of the two stimuli is indicated by the broken line (100%). *The decrease in amplitude of the response to the second stimulus resulting from delivery of the first stimulus was more marked after "late" depression by ACh.*

B (○——○), amplitude of the muscle action potential response to a single stimulus delivered after a train of 121 repetitive stimuli applied at a rate of 200 per second, plotted against the time interval between the end of the train and the single stimulus, before (left) and after (right) "late" depression by ACh. The amplitude of the response to the first stimulus of the train is indicated by the broken line (100%). *The increase in amplitude of the response to the single stimulus resulting from delivery of repetitive stimuli (post-tetanic facilitation) was more marked after "late" depression by ACh.*

tion of ACh was markedly inhibited during the "late" depression produced by ACh (Figs. 3, 9, and 12). This was more marked in the latter potentials of a train than in the initial potential. Doses (1 to 5 mg.) which produced an average of sixty-eight per cent "prompt" depression of the initial potential and sixty per cent of the fourth potential following the first injection of ACh, produced an average depression of twenty-two per cent and seven per cent during the "late" ACh depression. The degree of the inhibition increased with repeated injections of ACh and increasing "late" depression, so that doses of ACh several times that of the initial injection (up to 20 to 60 mg.) produced little or no "prompt" depression.

Reversal of the "late" ACh depression by ACh: The reparative effect of ACh

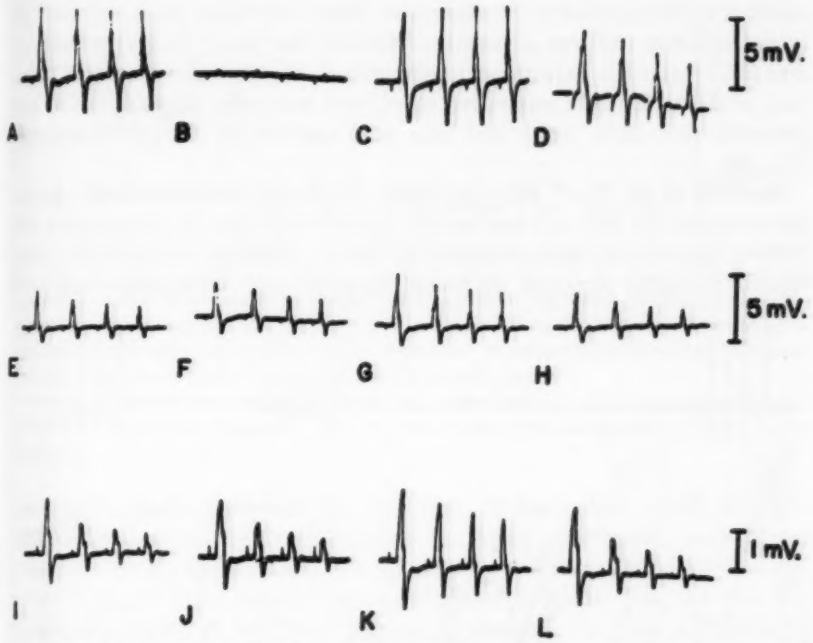

FIG. 12. "Prompt" and "late" depressant, and reparative effects of ACh on evoked potentials (upper row), and effect of ACh administered during "late" depression by ACh (middle row) or depression by choline (lower row). (Myasthenic patient, R. A.).

Upper row: Effect of the intra-arterial injection of ACh on muscle action potentials in response to a train of four nerve stimuli (40 msec. apart). A, control. B, "prompt" depression 10 seconds after 5 mg. ACh. C, repair 45 seconds after injection. D, "late" depression 3 minutes after injection.

Middle row: Effect of ACh administered after "late" depression produced by the prior injection of 5 mg. ACh four times in 45 minutes. E–H, as above, but there is now no prompt depression by ACh.

Lower row: Effect of ACh administered after depression produced by the prior injection of 100 mg. choline. I–L, as above.

During "late" depression produced by ACh, or depression produced by choline, there was inhibition of the "prompt" depressant effect of ACh, and increase in the reparative effect of ACh.

was usually increased during the "late" depression produced by ACh (Figs. 3, 9, and 12). The average amplitude (millivolts) of this repair was doubled, and, since the amplitude of the potentials was lower, the average per cent repair was three times as great. However, the maximum amplitude attained by the potentials after potentiation by ACh was usually below the amplitude present in the control state before the administration of any ACh. It seems

likely that this increase in the reparative effect above that which followed the initial injection was due to partial reversal of the "late" ACh depression. It was more marked in patients with mild myasthenia, in whom the initial injection of ACh had little reparative effect, and was most marked in the first potential of a train, which had been least repaired by the initial injection (Fig. 9).

Reversal of the "late" ACh depression by neostigmine: The "late" depression produced by ACh was reversed by neostigmine (Fig. 13). In patients with mild or moderately severe myasthenia, the administration of sufficient neosigmine promptly increased the potentials above their initial amplitude until

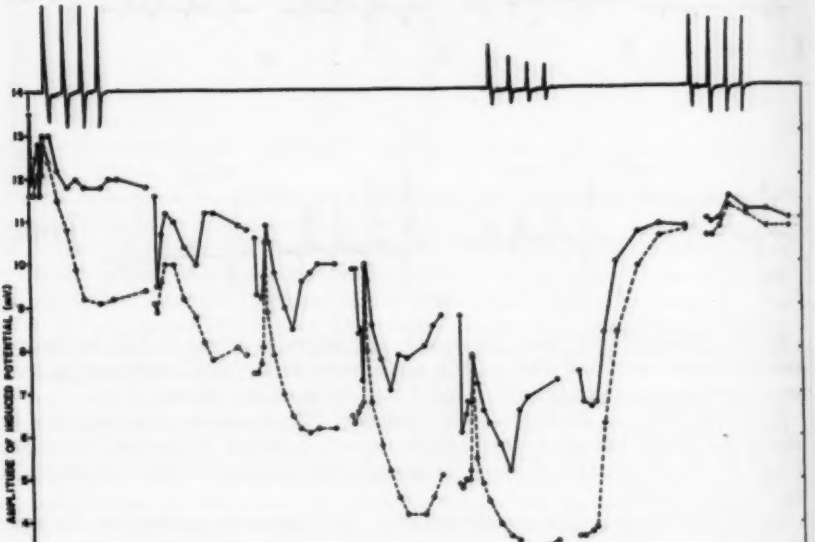

FIG. 13. Effect of neostigmine on the "late" depression of evoked potentials produced by ACh. The amplitude of the first (●——●) and fourth (○– – –○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds has been plotted.

Above: left, control muscle action potentials; middle, "late" depression after five intra-arterial injections of ACh; right, reversal of depression two minutes after intra-arterial injection of neostigmine. (Myasthenic patient, A. G.).

Neostigmine reversed the "late" depression produced by ACh.

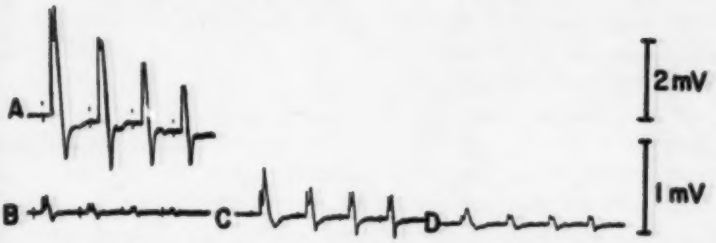

FIG. 14. Effect of neostigmine on the "late" depression of evoked potentials produced by ACh in a patient with very severe myasthenia gravis (R. S.). A, control muscle action potentials evoked by nerve stimuli 40 msec. apart. B, "late" depression following seven intra-arterial injections of 5 mg. ACh during 1½ hours. C, partial repair 30 seconds after the intra-arterial injection of 1 mg. neostigmine. D, depression 30 seconds after two additional intra-arterial injections of 1 mg. neostigmine, and 9 minutes after C.

In a patient with very severe myasthenia gravis, neostigmine produced only partial reversal of the "late" depression produced by ACh. Repeated injections of neostigmine resulted in further depression.

they approached or reached the amplitude attained when neostigmine was administered alone. The magnitude of the repair produced by neostigmine following "late" depression of the potentials by ACh greatly exceeded that which occurred when neostigmine was administered alone. This indicates that neostigmine reversed the "late" depression produced by ACh, in addition to all or part of the original myasthenic defect. In patients with severe myasthenia, the repair produced by neostigmine during "late" ACh depression was less complete, and repeated injections of neostigmine resulted in partial repair, followed by depression before the potentials reached the amplitude attained when neostigmine was administered alone (Fig. 14).

Effect of prior administration of neostigmine: The prior administration of neostigmine did not enhance the "late" depressant action of ACh; in fact, in some instances, there was partial inhibition of this action.

Lack of effect of atropine: Atropine sulfate, in doses of 1 to 3 mg. administered intramuscularly or intra-arterially, had no influence on the amplitude of muscle action potentials evoked by nerve stimuli, or on the reparative and depressant effects of ACh on these potentials.

Effects of Choline

The slow onset and the duration of the "late" depressant effect of ACh, as well as the failure of the prior administration of neostigmine to enhance or prolong this effect, suggested that it might be due to one of the products of hydrolysis of ACh. The injection of sodium acetate, in doses up to 200 mg., had no effect on the amplitude of muscle action potentials evoked by nerve stimulation. The administration of choline chloride, on the other hand, produced

slight transient increase in the amplitude of these potentials, followed by prolonged depression. The neuromuscular block produced by choline resembled the "late" depressant action of ACh, since it inhibited the "prompt" depressant action of ACh and was reversed by ACh or neostigmine.

Reparative effect: The administration of choline chloride, in doses of one to 100 mg., resulted in prompt, but slight increase in the amplitude of muscle action potentials evoked by nerve stimuli, in 12 of 23 patients (Figs. 15 and 16). This began 10 seconds after injection, was maximal in 18 seconds, and lasted about 30 seconds, being followed by depression of the potentials. Increasing doses up to 30 mg. produced increasing repair. Following larger doses the ensuing depression was predominant. The time course of the repair resembled that produced by ACh, but ACh was effective in smaller doses and its action was more uniform. Following choline, the initial potential of a train was usually increased as much, or more than subsequent potentials, while following ACh the latter potentials were increased to a greater extent.

Depressant effect: In those patients in whom choline produced transient re-

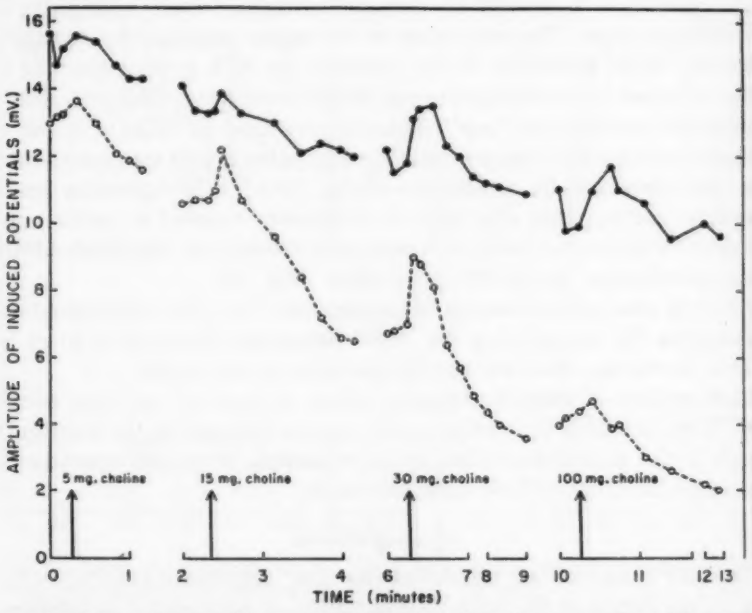

FIG. 15. Effect of repeated intra-arterial injections of choline on muscle action potentials evoked by nerve stimulation. The amplitude of the first (●——●) and fourth (○- - -○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every five seconds has been plotted. (Myasthenic patient, A. G.).

Choline produced transient repair, followed by depression.

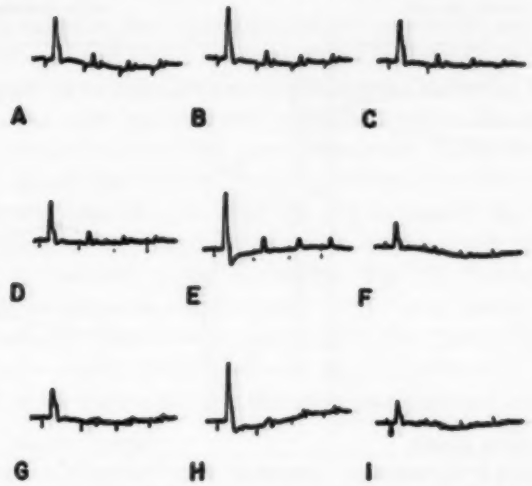

FIG. 16. Effect of repeated intra-arterial injections of choline on muscle action potentials evoked by nerve stimulation. (Myasthenic patient, R. S.).

Top row: Effect of 30 mg. choline on muscle action potentials in response to a train of nerve stimuli (40 msec. apart) evoked at five second intervals. A, control (amplitude of initial potential 2.6 mV). B, partial repair 20 seconds after injection. C, depression 5 minutes after injection.

Middle row: More marked effect of 250 mg. choline injected 3 minutes after C. D–F, as above.

Bottom row: Effect of 30 mg. choline injected 9 minutes after F, showing greater repair than occurred after the initial injection. G–I, as above.

Choline produced transient repair followed by depression, which was transiently repaired by the subsequent injection of choline.

pair of muscle potentials evoked by nerve stimuli, depression of these potentials began a mean of 30 seconds after injection and became maximal in 109 seconds, while in patients in whom there was no repair, depression began in 15 seconds and was maximal in 99 seconds (Table I). Recovery then occurred slowly over a period of 20 to 120 minutes. The initial potential of a train was usually depressed to a lesser degree than subsequent potentials, resulting in an increase in the decrement (Figs. 15 and 16). The depression produced by choline was partly and transiently reversed by the subsequent injection of choline, since the reparative effect of this drug, particularly on the initial potential of a train, was greater after depression by choline (Fig. 16).

The time course of the depressant effect of choline in the myasthenic patients differed significantly from that observed in normal subjects (Table I). The interval between injection of drug and onset of depressant effect was twice as long in myasthenic patients in whom choline produced no repair as in normal

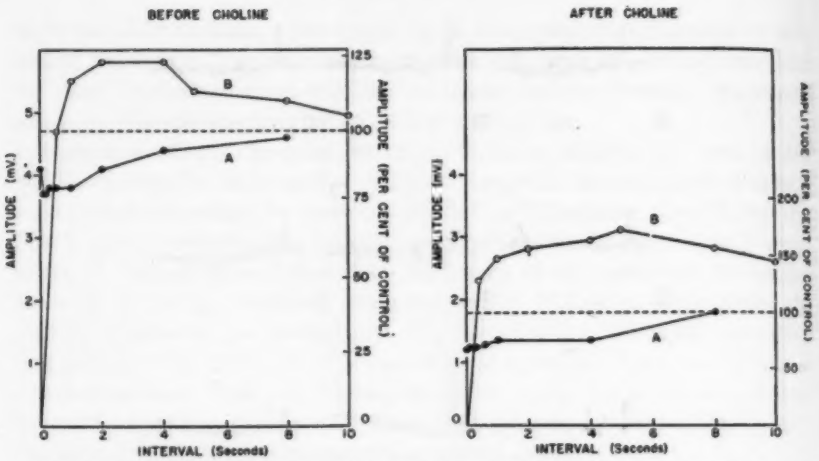

FIG. 17. Changes in the response of the muscle to nerve stimulation during the depression produced by choline. (Myasthenic patient, G. L.).

A (●——●), amplitude of the muscle action potential response to the second of two nerve stimuli plotted against the time interval between the two stimuli, before (left) and after (right) depression produced by the intra-arterial injection of 100 mg. choline. The amplitude of the response to the first of the two stimuli is indicated by the broken line (100%). *The per cent decrease in amplitude of the response to the second stimulus resulting from delivery of the first stimulus was slightly more marked after depression by choline.*

B (○——○), amplitude of the muscle action potential response to a single stimulus delivered after a train of 121 repetitive stimuli applied at a rate of 200 per second, plotted against the time interval between the end of the train and the single stimulus, before (left) and after (right) depression by choline. The amplitude of the response to the first stimulus of the train is indicated by the broken line (100%). *The increase in amplitude of the response to the single stimulus resulting from delivery of repetitive stimuli (post-tetanic facilitation) was more marked after depression by choline.*

subjects, and four times as long in patients in whom repair occurred. The time to maximal depression was four times as great, and the duration of the depression was about twice as great.

An increase in the degree of decrement occurred in myasthenic patients at all levels of choline block, in contrast to normal subjects, in whom decrement appeared only when the block was marked. The degree of depression of the initial potential of a train was the same in myasthenic patients as in normal subjects, while that of subsequent potentials was more marked in the former (Table II). The mean per cent reduction of the fourth potential of a train was two and a half times as great in myasthenic patients as in normal subjects, a significant difference. The difference in amplitude of reduction was not as great, and was not significant, probably because the amplitude of the latter potentials of a train prior to drug administration was much lower in myasthenic patients, particularly those with a severe degree of block.

As in normal subjects, the depressant effect of choline per mg. of drug was only one half to one third the "late" depressant effect of ACh.

Effect on response to repetitive nerve stimulation: Following depression by choline there was more marked decline (decrement) in the muscle action potentials in response to repetitive nerve stimulation. When a single or conditioning stimulus was delivered to the nerve and then a second or test stimulus applied at intervals from 16 msec. to 10 sec., the decrease in the muscle action potential response to the second stimulus was slightly more marked during choline block, as measured by per cent change (Fig. 17). Post-tetanic facilitation was moderately greater, as measured by per cent change.

Effect on "prompt" depressant action of ACh: *The "prompt" depressant action of ACh was markedly inhibited following the administration of choline* (Figs. 12 and 18). This was more striking in the latter potentials of a train than in the

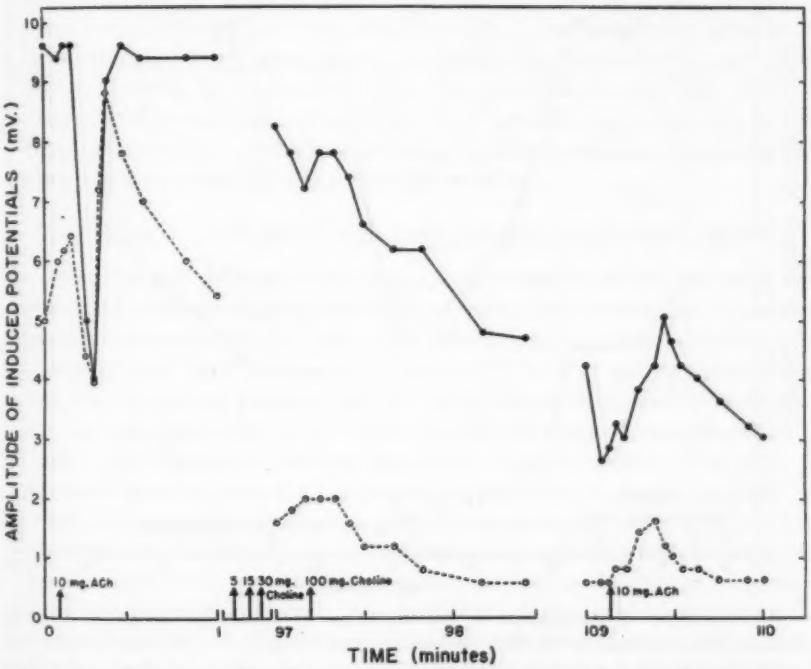

FIG. 18. Effect of ACh administered before and after depression of evoked potentials by choline. The amplitude of the first (●——●) and fourth (○– – –○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds has been plotted before and after intra-arterial injection of each drug.

During depression produced by choline there was inhibition of the "prompt" depressant effect of ACh, and increase in the reparative effect of ACh.

initial potential. The average "prompt" depression produced by 2 to 7 mg. of ACh after choline administration was 12 per cent of the first potential of a train and one per cent of the fourth, compared to 60 and 50 per cent before choline. The "prompt" depressant action of ACh on the fourth potential was completely blocked in 9 of 10 patients.

Reversal of choline block by ACh: In patients with mild myasthenia, in whom ACh, administered prior to choline, produced only slight increase in the

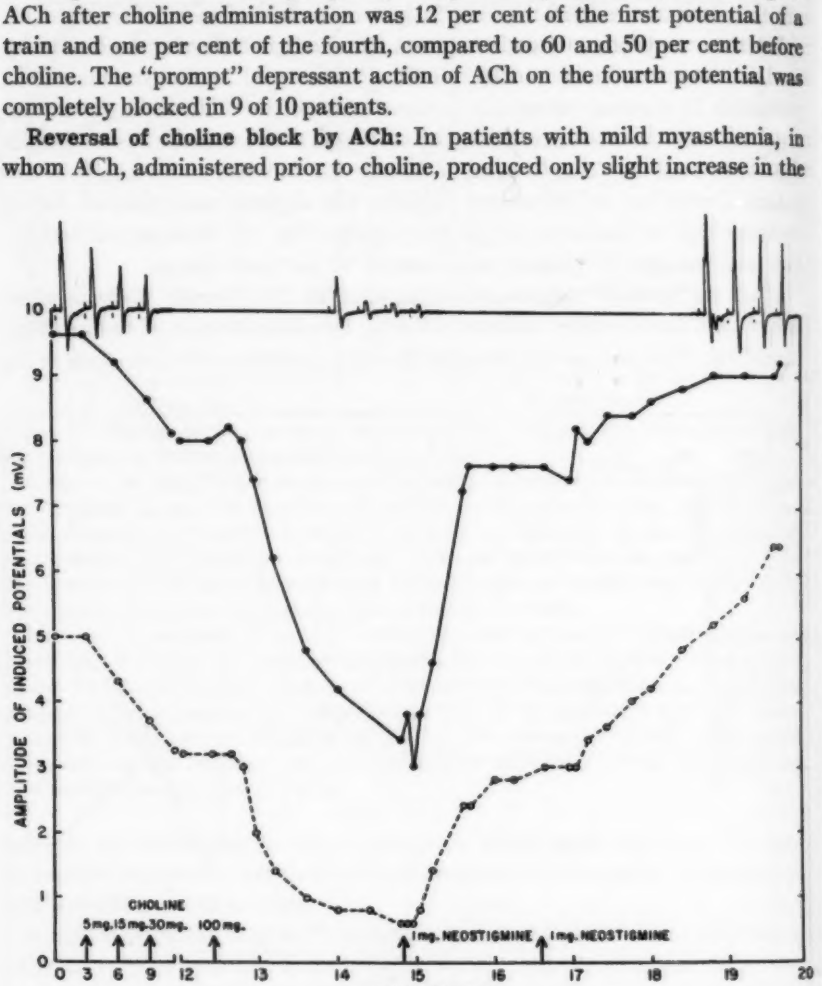

FIG. 19. Effect of neostigmine on the depression of evoked potentials produced by choline. The amplitude of the first (●——●) and fourth (○- - -○) muscle action potentials in response to a train of four nerve stimuli (40 msec. apart) evoked every 5 seconds has been plotted.

Above: left, control muscle action potentials; middle, depression 2½ minutes after the intra-arterial injection of choline; right, reversal of depression 7 minutes after the intra-arterial injection of neostigmine.

Neostigmine reversed the depression produced by choline.

amplitude of potentials evoked by nerve stimuli, the magnitude of the repair was greater after depression by choline (Fig. 12). In patients with severe myasthenia, ACh usually produced slightly more repair following choline of the response to the initial stimulus of a train, and less repair of the response to subsequent stimuli (Fig. 18).

The mean repair by ACh of the initial potential of a train was 0.6 mV prior to choline and 0.8 mV after choline, and of the fourth potential 1.1 and 0.4 mV. Since the amplitude of the potentials was lower after choline, the per cent repair produced by ACh was greater, being, on the average, 64 and 96 per cent repair of the first and fourth potentials, compared to 21 and 66 per cent prior to choline administration.

Reversal of choline block by neostigmine: Choline block was reversed by neostigmine, which produced a prompt and marked increase in the evoked potentials, usually to or above their initial amplitude (Fig. 19). In patients with moderately severe myasthenia the potentials attained the same amplitude as when neostigmine alone was administered. In patients with very severe myasthenia, repeated injections of neostigmine usually resulted in only partial repair, followed by depression before the potentials reached the amplitude attained following neostigmine alone. In almost all instances, however, the repair produced by neostigmine following choline depression was greater than that which occurred following neostigmine alone.

RESULTS IN PATIENTS WITH OCULOBULBAR MYASTHENIA GRAVIS

The effects of ACh on neuromuscular transmission in the clinically uninvolved *m. adductor digiti quinti* of 12 patients with oculobulbar myasthenia gravis were essentially the same as in normal subjects. The stimulating and "prompt" and "late" depressant effects of ACh in these patients were slightly less than in normal subjects, but the range was so wide that the differences are not significant (Fig. 1 and Table II). The influence of neostigmine on the "late" ACh depression was the same as in normal subjects. The mean depressant effect of choline was significantly less than in normal subjects. The degree of progressive depression of successive potentials evoked by repetitive nerve stimuli, the time course of this depression, and the effect of neostigmine on the depression, were the same in four patients as in normal subjects. In three other patients, however, the properties of the depression produced by choline were intermediate between those observed in normal subjects and in patients with generalized myasthenia gravis. In these patients, doses of choline as small as 30 mg. produced progressive depression of successive evoked potentials which was two to three times slower in onset than in normal subjects, and was reversed by doses of neostigmine (0.5 to 1 mg.) which normally intensified the depression.

DISCUSSION

Numerous studies have been carried out to compare the stimulating (twitch-producing) effect of intra-arterially administered ACh in normal and myasthenic subjects. Early observations suggested that myasthenic patients might have heightened motor responsiveness to ACh (4, 13), but subsequent reports indicated normal (14) or diminished reactivity (15, 16). In the present study, the range of reactivity was wide in both groups. The average was slightly lower in the myasthenic patients, but the difference was barely significant, and could not be correlated with the severity of the disease in the injected arm. While there may be a partial block to the stimulating effects of ACh in myasthenia, this technique is subject to such limitations that no firm conclusions can be drawn. Estimation of the smallest dose of ACh that will produce a twitch is difficult because of the frequent occurrence of some involuntary movement attributable to the pain produced by ACh. Furthermore, all but the most severely affected myasthenic patients show relatively slight evidence of neuromuscular block in response to a single motor nerve stimulus. The defect in neuromuscular transmission usually becomes pronounced only after nerve stimulation, whether voluntary or induced. Since the stimulating effect of ACh has always been determined with the extremity at rest, failure to demonstrate striking inhibition of this effect does not disprove that the defect in neuromuscular transmission in myasthenia gravis is due to inhibition of the action of ACh.

The "prompt" depressant effect of ACh on the initial response to a train of four stimuli was significantly less than normal only in those patients with severe weakness. The "prompt" depressant effect on the fourth potential was less than normal in almost all patients with generalized myasthenia, and was absent in those with severe weakness. Since the "prompt" depressant action of ACh is attributable to depolarization of the motor end-plates, these findings indicate that the defect in neuromuscular transmission in myasthenia gravis is associated with inhibition of the depolarizing action of ACh. The inhibition is demonstrable only when the myasthenic defect is appreciable, and varies with the degree of block. It could be due to inhibition of the action of ACh or to excessively rapid removal of this compound from the end-plates, as might occur in the presence of an excessive local concentration of cholinesterase. The latter possibility seems less likely, since the duration of the "prompt" depolarizing action of ACh in myasthenic patients, as indicated by the duration of the "prompt" depressant and reparative effects, was several times longer than that in normal subjects, as reflected by the "prompt" depressant effect, and was the same in patients with severe myasthenia as in those with a mild form of the disease. It is conceivable that inhibition of the "prompt" depressant effect of ACh on induced potentials in myasthenic patients could be due to deficient synthesis or release of endogenous ACh following

nerve stimulation, if the depressant effect were due to the combined action of injected and endogenous ACh. If this were the case, the injection of larger amounts of ACh should overcome any deficit in endogenous ACh and produce the same degree of "prompt" depression of all potentials as in normal subjects, but this did not occur. Therefore, the decreased "prompt" depressant effect of ACh in myasthenic patients appears to be due to inhibition of the action of this compound on the motor end-plates.

The "late" depressant effect of ACh on evoked potentials was more marked in myasthenic patients than in normal subjects, as measured by per cent reduction of the potentials, particularly the latter potentials of a train. The properties of the "late" depression were different in the two groups; in the myasthenic patients they were characteristic of a competitive type of block, in contrast to the non-competitive type that occurred in normal subjects. In the myasthenic patients there was an increase in the degree of progressive depression (decrement) of successive muscle action potentials evoked by repetitive nerve stimuli, while in normal subjects there was even depression. In myasthenic patients, but not in normal subjects, the "prompt" depressant (depolarizing) action of ACh was markedly inhibited during the "late" depression, particularly on the latter potentials of a train. The "late" depression was reversed to a slight extent following the injection of ACh, and to a greater extent following neostigmine.

The greater effect of neostigmine may be due to more ready access to the motor end-plate of endogenous ACh than of injected ACh, or to some action of neostigmine other than cholinesterase inhibition. It has been suggested that the reparative action of neostigmine on neuromuscular transmission in myasthenic patients may be due not only to its anticholinesterase activity, but also to a direct depolarizing action (17). However, it must be noted that the reparative action of neostigmine on evoked potentials was only slightly greater in most myasthenic patients than that of the initial injection of ACh. In addition, the maximum improvement in muscular strength produced by neostigmine in myasthenic patients is no different from that produced by other anticholinesterase compounds, such as tetraethylpyrophosphate (TEPP), octamethylpyrophosphoramide (OMPA), pyridostigmine (Mestinon), or ambenomium (Mytelase), to which no mechanism of action other than cholinesterase inhibition has been ascribed. These observations do not support the suggestion that neostigmine may have a "direct" effect on neuromuscular transmission in myasthenic patients.

The administration of choline resulted in a "prompt" transient increase in the amplitude of evoked potentials in most myasthenic patients, followed, in all, by prolonged depression. The initial effect is attributable to depolarization of the motor end-plates by choline, while the subsequent depression had

the properties of a competitive block, resembling the "late" depressant effect of ACh. The reduction in the latter potentials of a train evoked by nerve stimuli was more marked than that of the initial potential, and more marked than in normal subjects. The block inhibited the "prompt" depressant effect of ACh, and was reversed slightly by ACh and markedly by neostigmine. It had the properties of a competitive type of block at all levels in myasthenic patients, whereas in normal subjects mild or moderate degrees of block had the properties of a non-competitive type, and marked degrees those of a mixed type, both competitive and non-competitive. The onset of the depression was slower, and the duration longer, in the myasthenic patients.

The depressant effect of choline was less in clinically uninvolved muscles of patients with oculobulbar myasthenia than in normal subjects, but in some of these patients the type of block was intermediate between that observed in normal subjects and myasthenic patients. These observations suggest that there may be a latent defect in neuromuscular transmission in the clinically uninvolved muscles of some patients with oculobulbar myasthenia. There was no sharp dividing line in the type of block produced by choline in these three groups, but rather a gradual transition from normal subjects to patients with the most severe degree of myasthenia. Following depression by choline, or by the "late" effect of ACh, the induced potentials of patients with mild myasthenia, and of some patients with oculobulbar myasthenia, resembled those of patients with more severe myasthenia prior to drug administration, as indicated by the degree of progressive decline of successive potentials, by resistance to the "prompt" depressant effect of ACh, and by repair following ACh or neostigmine.

In patients with the most severe involvement of the hand muscles, intra-arterially administered ACh or neostigmine increased the amplitude of muscle potentials evoked by nerve stimuli, but these did not reach the normal range. The repeated injection of large doses of neostigmine resulted in progressive depression of the induced potentials before they attained normal amplitude. This phenomenon has its clinical counterpart in myasthenic patients who have only limited improvement following increasing doses of neostigmine, or of any other anticholinesterase compound (18). Further administration of anticholinesterase compound may result in a decrease in strength, with or without fasciculations. The cause of the limitation in the reparative action of anticholinesterase compounds is not known. The inability of such compounds to completely reverse the block produced by the injection of ACh or choline in patients with severe myasthenia suggests that at some motor end-plates the block is non-competitive in type; i.e., not reversible by ACh or neostigmine. At other end-plates the block is reversible and is probably competitive in type. In myasthenic patients who respond poorly to anticholinesterase compounds, it is likely that the block of most of the end-plates is of the non-competitive

type. In patients in whom excessive doses of anticholinesterase compound readily cause weakness, many of the end-plates may be normal.

It is not clear why ACh and choline produce a predominantly competitive type of block in myasthenic patients and a predominantly non-competitive block in normal subjects. Since decamethonium has similar effects (19, 20), it is likely that motor end-plates of muscles affected by myasthenia gravis may react abnormally to a number of compounds. Their abnormal response to ACh and choline is of particular importance because of the release of these compounds during the normal process of motor nerve activity. This response could be due to an alteration in the end-plate, or to the formation of an abnormal product of ACh or choline which has competitive blocking action. The longer latent period between the injection of choline and the onset and occurrence of maximum depression in myasthenic than in normal subjects is somewhat more suggestive of the latter. It is of interest that the choline ester of adipic acid having methyl groups on the nitrogen atom produces a depolarizing type of block in experimental animals, while the compound having ethyl groups on the nitrogen produces a competitive type of block (21).

Since the effects described above were produced by compounds which are normally released during neuromuscular activity, it is possible that the defect in myasthenia may be due to a competitive block produced by ACh, or by choline derived from its hydrolysis. The competitive type of block inhibits the depolarizing action of ACh more completely than the non-competitive type of block, which ACh produces in the normal subjects. There is evidence that ACh is continually released from the motor nerve endings in subliminal concentrations (22), which may account for the presence of some neuromuscular block prior to nerve stimulation in patients with myasthenia. While the time course and other properties of the "late" depressant effect of ACh suggest that this effect, and the neuromuscular block of myasthenia, are produced by choline released following the hydrolysis of ACh, the dose-effect relationships of the injected compounds are not entirely compatible with this. ACh produced three times as much depression as choline per mg. of injected drug. Since each mg. of ACh yields on hydrolysis 0.8 mg. of choline, one might expect ACh to have slightly less effect than choline. However, it is possible that ACh hydrolyzed at the neuromuscular junction by muscle cholinesterase may give rise to choline in closer proximity to the end-plates than would result from the injection of choline. ACh may penetrate more readily into the region of the end-plates by virtue of its ionic interaction with the receptor protein of the end-plate.

SUMMARY

The intra-arterial injection of acetylcholine (ACh) in patients with myasthenia gravis produced the following results:

1. Transient stimulation of motor activity attributable to depolarization of motor end-plates. This was less than in normal subjects, but the difference was barely significant.

2. "Prompt" transient depression of neuromuscular transmission, which appeared to be due to persistence of the initial depolarization of the end-plates. This depression was significantly less than in normal subjects, indicating that there is, in myasthenic patients, a competitive (ACh inhibitory) block to the depolarizing action of ACh.

3. Transient improvement in neuromuscular transmission. This did not occur in normal subjects.

4. "Late" depression of neuromuscular transmission which was more marked than in normal subjects and had the properties of a competitive type of block (progressive depression of successive potentials in a train, inhibition of the depolarizing action of ACh, and reversal of the block by ACh or neostigmine). This block did not appear to be due to ACh itself. Its time course, and resemblance to a comparable degree of block produced by choline, suggested that it may be due to choline released as a result of hydrolysis of ACh.

The relationship of these observations to an understanding of the defect in neuromuscular function in myasthenia gravis is discussed.

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FUNCTION AND DYSFUNCTION AT THE CARDIA[1]

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Swallowing is a complicated reflex that is, as it were, provided fully developed at birth; neither effort nor anxiety are expended on the process so long as it remains unimpaired. There are however, those who pervert it for kudos or cash. The medical student proverbially tries his skill at ingesting a pint of beer without swallowing, while the professional sword-swallower must be the envy of those who struggle in vain to pass an oesophagoscope beyond the cricopharyngeal sphincter. In the upright position we assume that food will go downward, yet we lower ourselves head first to drink out of a mountain pool without a fear that our recently swallowed lunch may reappear below us.

A part of the attraction of medicine is that the study of disorder brings with it an awareness of the normal that otherwise might be accepted without thought or comment. Further than this, it may provide a series of natural experiments, the correlation of which may help to reveal the designs of nature. The whole process of swallowing is a fascinating study, but for the present it is only possible to consider a part of it: that part concerned with the passage of food from the oesophagus to the stomach, for here particularly, disorders are common.

When food enters the stomach it should pass on into the duodenum and should never return into the oesophagus, but there are patients who are unable to prevent regurgitation, and there are also people who, for comfort or convenience, accept regurgitation almost as a natural process in themselves. A doctor who had been listening to a lecture such as this once admitted that the dullness of the discourse was alleviated for him by his ability to "chew his cud"; and that he had taken the precuation of having the sort of lunch that would taste as well "on return" as it did "on delivery".

An old Yorkshire woman confided that she had "ruminated" since childhood. "I allus went to chotch wiv a goodie i' me mooth, ah did ah seer, an ah chewed while we got to't psalms; then ah swallered it—but when t' sarmon came I could allus bring it back agin". "Aye", said her husband, "an its allus a mint, and a despert strang us an' all, it fair reeks all ower t'chotch".

It is, of course, inaccurate to refer to this process as "rumination" for the true ruminants do not regurgitate from the stomach into the eosophagus, but from the large squamous-lined rumen that is interposed between the oesophagus and stomach. A clear distinction must always be made between oesopha-

[1] A lecture under the auspices of The Herter Lecture Fund given November 23, 1954.

geal regurgitation, gastric regurgitation and vomiting, for their mechanisms and significance are quite different. If gastric regurgitation is so marked that food is returned to the mouth and swallowed a second time, and if this is habitual, then it may be called rumination, but this only for the sake of colourful description, and against the dictates of accuracy. A good circus may well include a man who can swallow goldfish and frogs and return them at will, and, if Mr. Mannix is to be believed, rats have been used for the same purpose. These tricks are the more easily understood after a long enough experience of sliding diaphragmatic hernia.

Much of our knowledge of the hiatus and the cardia has evolved from studies of the abnormal by radiology, oesophagoscopy and thoracotomy. Simple errors in the interpretation or the description of the findings have been so common that some of them might be mentioned now in order to dispose of them. In the radiographic examination of the oesophagus and stomach the pictures are those of the barium and not of the viscus. Sometimes a herniated stomach will be filled with barium in the recumbent position, and when the barium floods down below the diaphragm as the patient stands up, the radiologist may say that the hernia is reduced in the upright position. The hernia may in fact remain in the chest while the barium descends. It was to overcome this simple mistake that the Cushing clip was originally applied to the junction of oesophageal and gastric mucosa through the oesophagoscope, so that the movements of this area could be followed under the roentgen screen and correctly interpreted as movements of the viscus. A further point is that the true length of a stricture can only be gauged if both its faces are clearly outlined. Barium passing through a fine stricture may remain as a thin stream for some distance, even though it may lie in a broad channel. Unless the lower face of the stricture is shown by barium made to pass in the opposite direction it is not possible to deduce the length of the stricture. It is hardly necessary to emphasize that the most important part of the radiological examination of the oesophagus is the screening, and that a plate may be taken to suggest abnormalities that do not exist. If a peristaltic wave, driving barium before it, meets with a closed cardia, it may distend the lower oesophagus to look almost like a herniated stomach. This is the so-called "phrenic ampulla"; not an anatomical structure but a radiological appearance. It may collapse by emptying into the stomach, or, if the cardia remains closed it may burst upward, still further simulating a sliding hernia with regurgitation. Another pitfall is in the observations made "on inspiration" and "on expiration". The radiologist will tell the patient to take a deep breath and then to hold it while he takes a picture. This is described as a picture on inspiration. As far as the lungs are concerned it is in inspiration, but for the diaphragm it is more like expiration, for when the patient holds his breath in inspiration he immediately closes his glottis and relaxes his diaphragm. It is often quite difficult to persuade a patient to inspire

and hold himself actively in inspiration with an open glottis. If we are considering the effect of muscular contraction of the diaphragm on the barium column there is obviously room here for a mistake in description or deduction. The last point in the radiology of the oesophagus that is worth making is that oesophagitis may be intense but superficial and not demonstrable except by direct vision. The radiologist cannot detect superficial changes in the epithelium or changes from one type of epithelium to another. If he does so, direct observation through the oesophagoscope is as likely to prove him wrong as right. He may, from collateral evidence, suspect these things but he can do no more.

Oesophagoscopy itself has its own traps and pitfalls of which mention may be made of two only. The first is included with some misgiving for it involves criticism of the Jackson oesophagoscope. It might seem ungrateful to be critical when the Jacksons have contributed so lavishly to our knowledge of endoscopy but the Negus instrument gives a much better view, and is indispensable to the detailed investigation of fine changes at the lower end of the oesophagus. The second point is that when measurements are being made of the level from the incisor teeth at which changes occur they should be recorded both as the instrument is being inserted and as it is being withdrawn. As the oesophagoscope is being passed down it may easily reduce a herniated cardia ahead of it and the level of the gastric mucosa may appear normal. As the instrument is withdrawn however, the hernia may follow it so that gastric mucosa, first encountered at 41 cms. may be left behind at 37 cms. It is of the greatest importance that the exact level of any change in the wall should be carefully related to the levels anticipated from study of the radiographs if these are apparent. For example, the level of the gastric mucosa as seen through the oesophagoscope must be compared with the position of the cardia as seen on the radiograph, for if they do not correspond, it may be that a part of the oesophagus is lined by gastric mucous membrane. If a stricture or ulcer is found, biopsies of the mucosa both above and below the stricture should be taken where this is possible.

There is only one admonition that needs to be given about the findings at thoracotomy and that is that quite an extensive peptic stricture of the oesophagus may be present without any sign of it on the external surface of the organ or without its being even palpable. The reason for this is that usually the fibrous stricture is submucous and covered with perfectly normal muscle. If its exact level and its relation to the cardia have not been accurately determined by radiography and oesophagoscopy before operation, serious mistakes in surgical treatment may be made.

The normal anatomy of the cardia and hiatus have been described often enough, but emphasis may be placed on some of the relations that appear to be of clinical importance. The hiatus itself is a slit in the right crus of the dia-

phragm variably reinforced by slips from the left crus. It occurs in the posterior part of the diaphragm which in the body is vertical so that the oesophagus has to pass forward and to the left to reach the stomach. The boundaries of the hiatus are muscular except in the front where the fibres become partly tendinous and intersect to form a small raphé reaching from the hiatus behind to the central tendon of the diaphragm in front. Constant pressure in the hiatus, as may occur from a herniated stomach, may cause some atrophy of the anterior raphé, but more readily splits the muscular divarication behind and below. As the oesophagus passes through this short tunnel it is lightly held by the muscle itself but is more securely anchored by its fascial connections. The fascia propia is reflected from the oesophageal wall to become continuous with the fascia of the diaphragm, that layer which passes to the under surface being the stronger and being dignified by a special name, the phrenooesophageal ligament. This layer of fascia, important as it is in the repair of a hernia, has suffered by being given too pompous a name. Surgeons have been inclined to look for the sort of ligament that supports the inside of the knee joint, and, being disappointed, have denied its existence.

Operations on the cardia have shown that the description of an abdominal oesophagus 1½ inches long is inaccurate, and was probably based on dissection from the abdomen where traction had been used. The oesophagus enters the stomach immediately it emerges from the diaphragm so that the acute angle between the two is closely and completely occupied by diaphragmatic muscle.

The ability of the cardia to allow free passage of food downward whilst resisting reflux, remains a topic for argument. The position has not been improved by the desire to find a single factor that will explain everything. Indeed this quest has led to the suggestion that special muscle exists, perhaps even in the submucosa, that cannot be demonstrated by histology. There are those who assert that the mucosa is gathered up into a rosette which itself mechanically prevents reflux. This is surely putting the cab before the horse. Anyone old enough to have actually ridden in the cab knows that a mucosa-lined tube big enough to allow the passage of a mass will, when closing, form a rosette. Competence here does not depend upon the conformation of the mucosa but upon the muscular action which produces it.

The cardia depends for its efficiency upon more than one factor. The intrinsic muscle of the oesophagus and stomach, particularly the circular muscle of the former and the oblique muscle of the latter, must exert an influence on closure. The oblique angle of entry of the oesophagus into the stomach and the angulation of the lower oesophagus put it at a mechanical advantage for competence. The fascial attachments maintain its position in relation to the diaphragm, and the diaphragm itself not only compresses the oesophagus from side to side, but also by its contraction maintains and increases the angulation.

Thus where nature has the problem of passing a muscular tube through a muscular diaphragm for one-way traffic, the tube is bent, it is supported in position by fascial attachments, and a muscle sling is passed round it, compressing it and at the same time increasing its angulation. This general arrangement is seen at both the oesophago-gastric and ano-rectal junctions, although it must be noted that this has been criticized by one surgeon on the ground that the sphincter at the lower end of the intestinal canal "has a much greater sense of responsibility".

The above conception of the factors contributing to competence of the cardia is based on many and diverse observations during life, of which the following are the most important: if the fascial connections of the lower oesophagus are stretched so that the cardia rides up into the mediastinum, reflux is inevitable: if the muscle of the lower oesophagus and adjacent part of the stomach is cut longitudinally and completely, so that the mucosa bulges freely, no reflux occurs so long as the fascial connections are intact and the cardia is returned to its proper place in relation to the diaphragm. This is done as a routine surgical treatment for cardiospasm. If such a myotomy is combined with lateral section of the fibres of the right crus which form the left border of the hiatus, incompetence and reflux occur. This reflux can be cured by careful suture of the divided muscle and repair of the fascia. If cardiospasm is associated with either a sliding hernia or with prolapse of the cardia into the abdomen, longitudinal division of the oesophageal and gastric muscle produces reflux, but if such a myotomy is combined with repair of the fascia and reconstitution of the right crural sling, competence is retained.

If we accept that nature through the ages has found the best way to control the cardia we can hardly do better than try to imitate it when we have to repair an abnormality here. The shortening of the stretched ligaments, their suture to the fascia on the under aspect of the diaphragm, the excision of excess fat from the hiatus, and the reapposition of the muscular fibres of the crus without strangling them would appear to be the nearest approach to what nature designed. This has already been described, and has been performed often enough by many surgeons to have become acceptable for those patients whose tissues still have some youthful resilience.

The hernia most commonly occurring at the hiatus is that where the cardia slides upward into the mediastinum, stretching its weakened ligaments and relaxing the oesophagus in its length. This sliding hernia is associated with reflux of gastric and bilious fluid into the oesophagus. In very many patients it appears to do no great harm, for they have no symptoms, and the lining of the oesophagus remains intact. Whether such immunity is dependent on a good secretion of mucus from the mouth, pharynx and oesophagus, or some unknown immunity of the tissues themselves, or to both is unknown. Certainly many unsuspected herniae are found on routine examinations for other

conditions, and some may cause only very mild symptoms that can easily be tolerated when their cause is known. These do not need operation. They should be warned against putting on too much fat or too tight clothes, and they should walk rather than slouch in a motor car or arm chair. The head of the bed may be raised a little at night, but sleeping in a propped-up position is not satisfactory, for bending forward at the waist compresses the abdomen and relaxes the diaphragm.

Other patients with this abnormality may complain of severe pain indistinguishable from that of ischaemic heart disease, and this independently of oesophageal ulceration. Some have attacks of dysphagia, often with complete obstruction where severe spasm or corkscrew contractions may be shown by the radiologist. Both the symptoms and the abnormal contractions may be relieved for a time by oesophagoscopy. This is quite distinct from the obstruction of ulceration and fibrosis.

Very many patients complain of flatulent dyspepsia, heartburn and acid regurgitation, and some of these, more especially the men, may advance to chronic ulceration and fibrous stricture. The patients selected for operation are those with symptoms or those in whom oesophagoscopy shows erosions in the mucosa. Operation should be preceded by a weight-reducing diet, the disposal of all stays, whether of steel, whalebone, or tight elastic, and in men by the replacement of belts by the shoulder suspension of trousers. The gravity of symptoms in relation to possible operation should be judged at the end of such medical measures. Many patients, previously judged suitable for operation, will then be found to be happy and free from trouble. Another precaution before the final decision is the investigation of the rest of the stomach and duodenum for ulceration, and of the gall-bladder for dysfunction or stones.

A second type of hernia at the hiatus is the "rolling" or para-oesophageal hernia. The characteristic feature here is a rolling-up of the anterior surface of the stomach into a peritoneal pouch in the mediastinum situated in front of the oesophagus. The great omentum and the transverse colon may be dragged up also until the stomach lies almost upside down in the chest. The most feasible explanation for this type of hernia is that a preformed hernial sac has existed since birth. This hypothesis has the support of the embryologists. The cardia may remain fixed in its normal position but more usually slides up to some extent. Reflux into the oesophagus however does not occur because the angulation between oesophagus and stomach is increased, and the distended stomach between the heart and the spine compresses the lower oesophagus. It is not unusual for these factors to prevent the entrance of the oesophagoscope into the stomach, and more harm than good may result from too much perseverance in this direction. Patients with these herniae may suffer from anginal pain, breathlessness or anaemia, but they do not have oesophagitis or stricture of the oesophagus. The herniated stomach may become ulcer-

ated, particularly at the point where it passes through the diaphragm, but such ulcers will often heal spontaneously after reduction of the hernia. Local excision may be thought advisable at the time of operation but partial gastrectomy should not be performed. The repair of the hiatus is the same as for sliding hernia. It may need to be reinforced a little more in front owing to greater atrophy here from the bulk of the hernia, the aim always being to leave the oesophagus in its natural position in relation to the diaphragm.

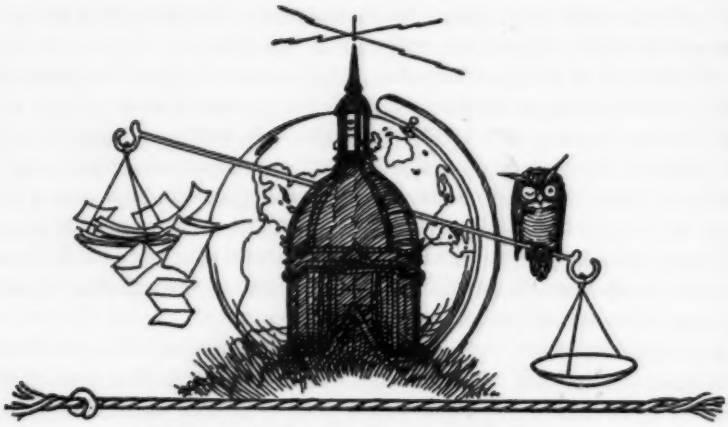
The presence of gastric mucous membrane lining the lower part of the oesophagus introduces a complication into both the diagnosis and the treatment of hiatal hernia. It may occur in association with any of the varieties, and has been described by others without a hernia. The gastric lining sometimes extends for only a few centimeters up the eosophagus or it may reach the aortic arch. It is usually of cardiac type. Not only can oesophageal mucous glands be seen in it, but quite large islands of stratified squamous epithelium may also be present. Oxyntic cells are rarely seen. The histologic picture does not suggest that oesophagitis, when it exists, is the result of the secretion from the gastric epithelium that lines the oesophagus, but rather from that in the stomach itself. When the outside of such an oesophagus is viewed through a thoracotomy it is not possible to say which part is lined by one kind of epithelium and which by another. That lined by gastric mucosa may feel slightly thicker, but this is not at all reliable. It has already been stressed that the stricture from peptic oesophagitis may not be palpable from the outside. The possibilities of error that occur when a patient has a stricture and a mixed epithelial lining are therefore great and are well illustrated by the following: a fat middle aged woman complained of dysphagia. Oesophagitis and a stricture were found 25 cms. from the alveolar margin. Immediately below the stricture was gastric mucous membrane. At operation the cardia was only 4 cms. above the hiatus which in this patient would be about 35 cms. from the alveolar margin. The oesophagus above the cardia looked and felt normal. If the level of the stricture, which corresponded with the aortic arch, had not been carefully noted at oesophagoscopy, it would have been assumed at operation that it was immediately above the stomach. A resection at this level would have left the stricture behind. Alternatively, if the true condition had not been observed personally through the oesophagoscope, it might well have been doubted that a stricture was present at all. A simple reduction of the hernia would have left the patient no better off.

When chronic ulcers occur in the gastric, as distinct from the squamous mucous membrane lining the oesophagus, they are more often palpable and more often associated with changes spreading into the oesophageal muscle and through this into the mediastinum. This is not always so however, for in one personal case a patient was subjected to oesophagectomy for stricture and severe bleeding, and only when the specimen was opened were two ulcers

found, one in the squamous epithelium causing stenosis, and one below the stricture in the gastric epithelium which had bled but which could not be seen at oesophagoscopy and was not palpable at operation.

In the absence of stricture formation the presence of a gastric mucosal lining to the lower oesophagus complicates the reduction of a hernia to form a competent cardia. Success with this is not impossible, but experience shows that such patients are more likely to be left with incompetence even though the stomach is shown to be in the abdomen. If the original disability was a sliding hernia the failure to produce competence is disappointing enough, but if it was a para-oesophageal hernia the reduction of the stomach to the abdomen without a competent cardia leaves a patient much worse off than before, as he is now liable to oesophagitis.

The presence of gastric epithelium in the oesophagus therefore should make the surgeon tread warily. Perhaps this implied suggestion that a surgeon use his feet may be resented by those whose pride is in their hands, but there are times when a good walk causes less heartburn than an ill-judged operation.



BOOK REVIEWS

Mental Health Planning for Social Action. By GEORGE S. STEVENSON. 358 pp., $6.50. The Blakiston Division, McGraw-Hill Book Co. Inc., New York, N. Y.

Perhaps the main value of this book by the nation's, perhaps the world's, most experienced student of programs for the promotion of mental health is its demonstration of the interpenetration of the field into a very large number of the social institutions that make up a culture. Its greatest fault is by no means unique in books in the field; it raises many questions without answering them, and it opens vistas for important work but offers few paths for reaching the not-too-distinct goals. By clarifying the questions and setting some goals, the book does an important service, nevertheless.

Part one deals with the diversity of agencies in the field and with the issues of program planning. The goals of social action are three; the restoration of mental health, the preservation of mental health and the elevation of mental health. A set of six criteria applicable for the judgment of programs is set forth and should prove a helpful list; although few offer any basis for more than subjective judgment in evaluating programs against the criteria, they offer a pattern for orderly thinking that will be helpful.

The next three sections deal in detail with the goals of social action listed above. The types of institutions treating psychiatric patients are discussed in detail. The chapter on Psychiatry in the General Hospital is the best marshalling of the issues here concerned in the literature. The discussion of facilities for the mentally deficient reveals in stark outline the deplorable lack of coordinated services in this area of growing public and professional concern. Although Stevenson has raised his former estimate of 8 per cent of mentally deficient cases as due to clear brain pathology to 14 per cent, he does not mention recent important epidemiological researches in this field implicating obstetrical problems, including prematurity, in the etiology of mental deficiency. There is a helpful discussion of the newer day and night hospital practices.

Under the protection of Mental Health, there is but one chapter, relating public health practices to mental health.

The Section of the Elevation of Mental Health is a review of social institutions of impor-

tance in reaching the goal. The material exceeds that indicated by the chapter titles. There is an excellent discussion of the relation between the health of the family relationships and the mental health of the individuals belonging to the family.

The final section deals with clarifying the issue of need in a particular area and an introduction to agencies operating in international efforts to improve mental health.

Although the bibliographies are not at all comprehensive, the fact that the book represents the vast experience of the author will make it a value reference work for all interested in social psychiatry and in planning mental health programs.

P. V. L.

Annual Review of Medicine, Vol. 7, 1956. Edited by DAVID A. RYTAND. 611 pp., $7.00. Annual Reviews, Inc., Stanford, California.

This volume, like its predecessors, tries to present a panoramic view of modern medicine. Its basic plan is at the same time its greatest strength and its greatest weakness. The twenty-five chapters cover a tremendous variety of topics, and while any physician will find sections of considerable interest and value, most doctors will also be able to find chapters that are dull and uninviting. Since this reviewer does not pretend to be a Renaissance figure, it is difficult to be sure whether the "failures" to be described are to be blamed primarily on the authors or on the reader, but my own score-board reads as follows: a) twelve excellent to good chapters, with particular kudos for the endocrinology chapter by Dobbs, Garrod, and Simpson; the cancer chapter by Laszlo, Spencer, and Weiss; and the chapter by Sweet on the diseases of the nervous system, b) six "adequate" chapters (which might have seemed better if the topics had been more interesting to the reviewer), c) four poorly written chapters, consisting essentially of consecutive "capsule" summaries of articles, with no attempt at graceful integration, and d) three chapters that would have been better left unpublished. Particularly objectionable is the chapter on "Advances in the Treatment of Sterility" by Farris. It is hard to believe that a review of this field should have only sixteen references, fourteen of which are to the work of the author and his colleagues. In addition it seems in questionable taste for the author to use this chapter as an advertisement for his "Conceptulator" (complete with manufacturer's name and address!). An editorial error was also made in assigning the chapter on sympathetic blocking agents to a pharmacologist working for a pharmaceutical firm with very definite reasons for enthusiasm for reserpine and certain ganglion blockers. Since there are 564 pages in the book, and approximately half these pages will probably be of interest to any given physician, the price seems reasonable indeed.

LOUIS LASAGNA

Diseases of the Chest. By H. CORWIN HINSHAW AND L. HENRY GARLAND. 727 pp., $15.00. W. B. Saunders Co., Philadelphia, Pa.

The authors, who are clinical professors of Medicine and Radiology at Stanford University, have collaborated to make this book one of outstanding merit. The discussions are not focussed too sharply on the chest, the point being made repeatedly that a complete examination, using all the means at our disposal, is necessary if we are to have an adequate comprehension of any disease. The text is illustrated liberally with x-ray pictures of superior quality, but the authors are careful to point out the danger of relying too heavily on this diagnostic method. Relatively few references are included, but the ones used are chosen carefully.

Some of the high points seem worthy of especial emphasis. The segmental anatomy of the lungs and bronchial tree is discussed and illustrated in great detail; this should serve as a valuable reference source rarely found in standard text-books. The various problems relat-

ing to the pathogenesis and physiological changes in emphysema are presented clearly, and the chief functional studies outlined in a concise manner. A large section is devoted to tuberculosis; here the point is made that each case is a separate problem, demanding an intensive program of investigation and treatment. The place of collapse therapy in any such regimen is evaluated; it is their opinion that the procedure—especially pneumoperitoneum—may still be indicated in many cases and should not be replaced entirely by specific drugs. The section dealing with drug regimens contains a critical evaluation based on a consideration of long-term experiences and present trends. In patients undergoing surgery it is the authors' opinion that prolonged periods of pre and post-operative medical therapy are of major importance. A consideration of the long-term results seems to strengthen this conclusion. Occupational pulmonary diseases are discussed in the last chapter. This material should serve as an excellent reference source for anyone requiring exact data on the degree of exposure that may be dangerous, the nature and extent of the pulmonary changes, and whether or not they are compensable.

A. M. FISHER

Counseling in Medical Genetics. By SHELDON C. REED. 268 pp., $4.00. W. B. Saunders Co., Philadelphia, Pa.

The author states that this book was written for the guidance of the practicing physician in counseling patients with problems of hereditary disease. It is oriented by the common inquiries presented to the Dight Institute for Human Genetics (University of Minnesota) of which the author is Director. There are introductory chapters of simple explanation of genetic laws and some tools such as studies of twins and consanguinity. Following this are individual chapters on common traits which have an incidence of one or more per 1000 births, including mongolism, nervous system anomalies, clubfoot, harelip and cleft palate, congenital heart disease, congenital dislocation of the hip, mental retardation, schizophrenia, manic-depressive psychosis, diabetes, allergies, tuberculosis, etc. In some of these chapters, the evidence and discussion seems fragmentary, a fault which may arise from the author's effort to keep the book small. This is remedied partially by frequent modern references to studies which the reader may consult on any disease of special interest to him. The rare traits, many of which have a more definite genetic background than some common traits, are treated in an appendix adapted from Fraser. There are 196 entries from acatalasia to veroderma pigmentosum with a few lines on each as to type of inheritance and frequence of occurrence.

The author is (justly) critical of uninformed genetic advice by physicians, but many medical readers may have a reciprocal feeling about oversimplified medical advice by geneticists as evidenced in some of the illustrative examples of counseling at the end of each chapter. This small volume contains a good deal of information though initially the reader may be misled by the casual style in which it is written.

E. D.

The Neuroanatomical Basis for Clinical Neurology. By TALMAGE L. PEELE. 564 pp., $12.50. McGraw-Hill Book Co., New York, N. Y.

This text is written in a clear, readable fashion. It is actually a composite, including descriptive neuroanatomy, the principles of neurophysiology and experimental investigations, continually interweaving the application of these to clinical neurology.

The author initiates the reader by presenting in the first chapter the fundamentals of the unit structure of the central nervous system, the neuron and its processes. The second chapter, in a similar fashion, presents the current concepts regarding the supportive struc-

tural units, namely the neuroglial and microglial elements. By the very nature of this subject material there is considerable histoanatomical discussion. In the third chapter the author sees fit to present the embryological development of the central nervous system in a very brief manner. Chapters 4, 5 and 6 present in a cursory manner the gross anatomy of the brain, spinal cord and peripheral nerves. These chapters have the material well arranged and devote more than the usual space to the blood supply and drainage, a subject which is currently receiving more and more attention. The autonomic nervous system receives considerable emphasis in Chapter 7, which presents the general anatomical features, and later in Chapter 14, in which the physiological and clinical features of the hypothalamus are discussed. The special sensory pathways are clearly presented in the usual fashion.

The author accomplishes his purpose of attempting to integrate the anatomical, physiological and clinical approach to studying the central nervous system. In spite of such a complex objective, the author avoids confusing the reader by omitting the speculative or uncertain concepts. The reader is aided by a generous sprinkling of well placed diagrams and drawings and photographs of human brain sections.

Due to the general treating of the central nervous system the text, of necessity, fails to present much of the necessary details for each of the treated spheres. The bibliography is adequate for ready reference.

The text can be recommended for enjoyable and useful reading for the medical student and practicing physician.

GEORGE W. SMITH

BOOKS RECEIVED FOR REVIEW

Adrenal Function in Infants and Children. A Symposium. Edited by LYTT I. GARDNER. 221 pp., $6.75. Grune & Stratton, Inc., New York, N. Y.

Atlas of Exfoliative Cytology. Supplement 1 (Looseleaf). By GEORGE N. PAPANICOLAOU. $4.00. Published for The Commonwealth Fund by the Harvard University Press, Cambridge, Mass.

Clinical Orthopaedics No. 7. Edited by ANTHONY F. DEPALMA. 354 pp., $7.50. J. B. Lippincott Co., Philadelphia, Pa.

Electrodiagnosis and Electromyography. Vol. 1 of Physical Medicine Library. Edited by SIDNEY LICHT. 272 pp., $10.00. Elizabeth Licht, Publisher, New Haven, Conn.

Endogenous Uveitis. By ALAN C. WOODS. 303 pp., $12.50. The Williams & Wilkins Co., Baltimore, Md.

Fifth Annual Report on Stress, 1955/56. Edited by HANS SELYE AND GUNNAR HEUSER. 815 pp., $20.00. MD Publications, Inc., New York, N. Y.

Gynecologic Nursing, 5th edition. By ROBERT JAMES CROSSEN AND ANN JONES CAMPBELL. 269 pp., $4.25. The C. V. Mosby Co., St. Louis, Missouri.

A History of the Therapy of Tuberculosis and The Case of Frederic Chopin. By ESMOND R. LONG. 71 pp., $2.00. University of Kansas Press, Lawrence, Kansas.

Hormones and the Aging Process. Proceedings of a Conference held at Arden House, Harriman, New York, 1955. Edited by EARL T. ENGLE AND GREGORY PINCUS. 323 pp., $8.50. Academic Press, Inc., New York, N. Y.

Human Ovulation and Fertility. By EDMOND J. FARRIS. 159 pp., $6.50. J. B. Lippincott Co., Philadelphia, Pa.

Laboratory Manual in Physiology and Anatomy. With Study Guide Questions and Practical

Applications. By ELLEN E. CHAFFEE. 219 pp., $2.00. J. B. Lippincott Co., Philadelphia, Pa.

A Manual of Anesthetic Techniques. By WILLIAM J. PRYOR with a foreword by J. H. T. CHALLIS. 224 pp., $7.00. The Williams & Wilkins Co., Baltimore, Md.

Medicinal Chemistry, Vol. II. Edited by F. F. BLICKE AND C. M. SUTER. 311 pp., $10.00. John Wiley & Sons, Inc., New York, N. Y.

Meditations on Medicine and Medical Education Past and Present. By I. SNAPPER. 138 pp., $3.75. Grune & Stratton, New York, N. Y.

The Mentally Retarded Patient. By HAROLD MICHAL-SMITH. 203 pp., $4.00. J. B. Lippincott Co., Philadelphia, Pa.

Microbiology and Pathology, 6th edition. by CHARLES F. CARTER AND ALICE LORRAINE SMITH. 970 pp., $6.50. The C. V. Mosby Co., St. Louis, Missouri.

Mosby's Review of Practical Nursing. Edited by DOROTHY KELLEY RAPIER, MARIANNA JONES KOCH AND DEBORAH MACLURG JENSEN. 322 pp., $4.25. The C. V. Mosby Co., St. Louis, Missouri.

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